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SPECIES INTERACTIONS AND METABOLIC DEPENDENCIES IN THE HUMAN GUT MICROBIOTA

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List of abbreviations

16S rRNA	16S ribosomal RNA
ASV	Amplicon Sequence Variants
Bact1	*Bacteroides* 1 enterotype
Bact2	*Bacteroides* 2 enterotype
BC	Bray-Curtis
CB	Cary Blair
CDI	*Clostridioides difficile* infections
CP57	Citrus pectin
dbRDA	Distance-based redundancy analysis
DMSO	Dimethyl sulfoxide
Eu	Euclidean
FGFP	Flemish gut flora project
FMT	Faecal microbiota transplantation
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GI	Gastro-intestinal
GLMs	Generalized linear models
GMMs	Genome metabolic modules
GSMM	Genome-scale metabolic modelling
GTDB	Genome Taxonomy Database
HAEC	Health-associated enterotype cores
HAEFC	Health-associated enterotype functional cores
HAFC	Health-associated functional core
HPLC	High-performance LC
IBD	Inflammatory bowel disease
IMG	Integrated Microbial Genome Database
KO	KEGG orthology
LB	Luria-Bertani
LC	Liquid chromatography
MALDI-TOF MS	Matrix assisted laser desorption ionization-time of flight mass spectrometry
mGAM	modified Gifu Anaerobic Medium
MS	Mass-spectrometry
NMR	Nuclear magnetic resonance spectroscopy
ONT	Oxford Nanopore Technologies
OTUs	Operational taxonomy units
PacBio	Pacific Biosciences
Prev	*Prevotella* enterotype
RCM	Reinforced clostridium medium
RDP	Ribosomal Database Project
RID	Refractive index detection
Rum	*Ruminococcus* enterotype
SCFAs	Short-chain fatty acids
UPLC	Ultra-performance LC
VANISH	Volatile and/or Associated Negatively with Industrialized Societies of Humans
WC	Wilkins-Chalgren
WGS	Whole genome sequencing

Summary

The human gut microbiota is essential for host well-being, and various diseases have been linked to a dysfunctional microbiota. However, there is still a lack of in-depth understanding of how gut communities function as an ecosystem, which can hinder efforts to unravel the aetiology of diseases and develop effective therapeutics to modulate the gut ecosystem. One promising approach is to use simplified communities through either a bottom-up or top-down approach. The former involves assembling isolated strains into a community, while the latter entails reducing a complex community into a simpler one using culture-based enrichment. This thesis aimed to use simplified communities to understand bacterial interactions and develop a framework for designing future biotherapeutics to modulate the human gut microbiota.

First, the study involved building an in-house culture collection using different cryopreservation conditions of stool samples to evaluate their effect on anaerobic bacterial isolations. The results showed that cryopreservation formulations influenced the richness and taxonomic diversity of gut anaerobes recovered from the faecal samples. The parallel use of standard dry freezing and supplementation with DMSO was found to increase the number of recovered species indicating that the two conditions were complementary.

Then, the study aimed to design health-associated enterotype functional cores of the human gut microbiota based on both the abundance and prevalence of members within a healthy cohort in addition to differences between enterotypes. The selected core species from the *Bacteroides* 1 and *Prevotella* enterotypes were further characterised to define their metabolic capabilities using in silico analysis and in vitro data from the literature. The findings provided a deeper understanding of the ecological niche of the core species and their potential competitive and cooperative interactions at the community level.

For the bottom-up approach, two minimal health-associated enterotype functional cores were assembled in vitro based on the selected health-associated enterotype cores using strains mainly from the in-house culture collection. Co-cultures and small communities were tested to investigate potential competition and cooperation features. The results showed that four-member communities based on functional complementarity enabled members of the community to coexist. The resulting community of about 10 members was resilient to biotic and abiotic perturbations. This study provided valuable insights into designing future biotherapeutics, and contributed to a better understanding of community dynamics and interactions in the human gut microbiota.

During the top-down approach, a series of repeated batch fermentations were carried out to assess the community dynamics using two stool donors belonging to two enterotypes, *Prevotella* and *Bacteroides* 1, from healthy volunteers in the presence of various substrates. The study revealed that enriched communities were inoculum-specific rather than driven by medium only, with taxon-substrate associations that could be further explored for targeted isolation of specific taxa. The results highlight the importance of enriching complex gut communities in different nutritional conditions to better understand metabolic interactions in the human gut.

While both come with their specific limitations, the bottom-up and top-down approaches were found to be valuable for studying interactions at different levels of complexity. In the future, an iterative approach using the top-down approach to increase knowledge on species interactions and the bottom-up approach to build a well-defined community will be necessary to target specific community compositions or functions towards building next-generation probiotics.

Chapter I

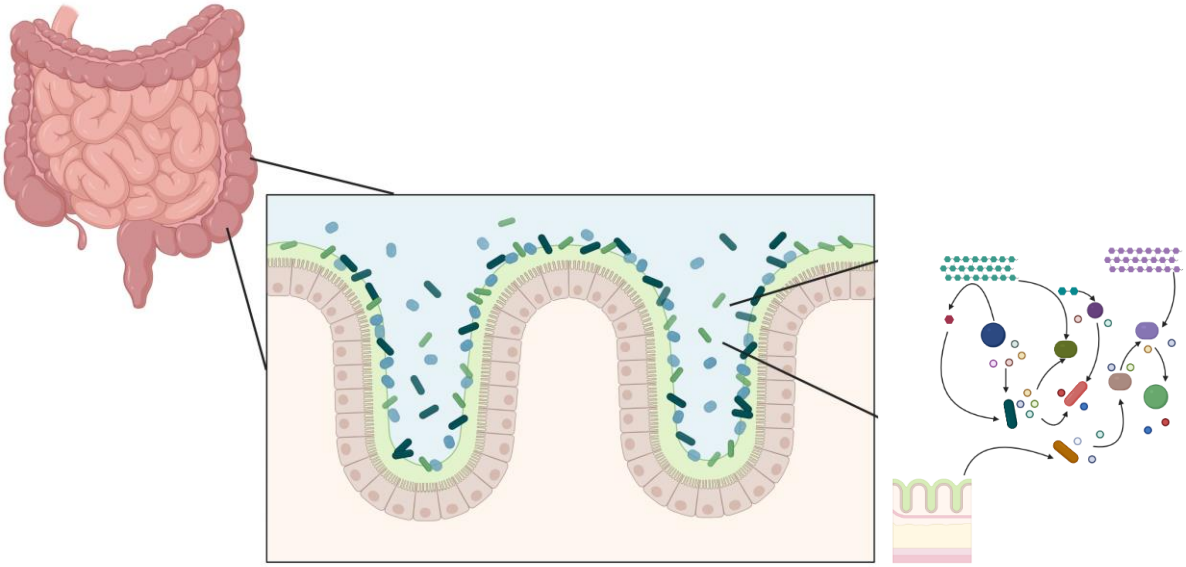
General introduction

&

Objectives

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*contributed equally.



Introduction

Human gut microbiota composition

The human gastro-intestinal (GI) tract, comprised of the oesophagus, stomach, small and large intestine (colon), harbours a diverse array of microorganisms, from the oral cavity to the rectum. This collection of bacteria, archaea, small eukaryotes and viruses, commonly referred to as the GI microbiota, constitutes a highly complex and interactive microbial ecosystem[2]. The interplay of these microorganisms not only structures[3], stabilises[4], and modulates[2] the diversity of microbial communities, but also contributes to host physiological homeostasis[5]. Physicochemical parameters, such as transit time, oxygen and pH, as well as diet and host-generated antimicrobial peptides, play a crucial role in shaping microbial diversity within the GI tract (Figure 1). The microbial density is largely concentrated in the colon, where the pH is gradually increasing (between 5.2 and 7) and there is an oxygen gradient from the mucosal layer to the lumen, allowing different microorganisms to occupy distinct ecological niches[6]. It has been shown that a bacterial population of 10^{12} bacteria/g of faecal matter can express 500 times more genes than human protein-coding genes[7].

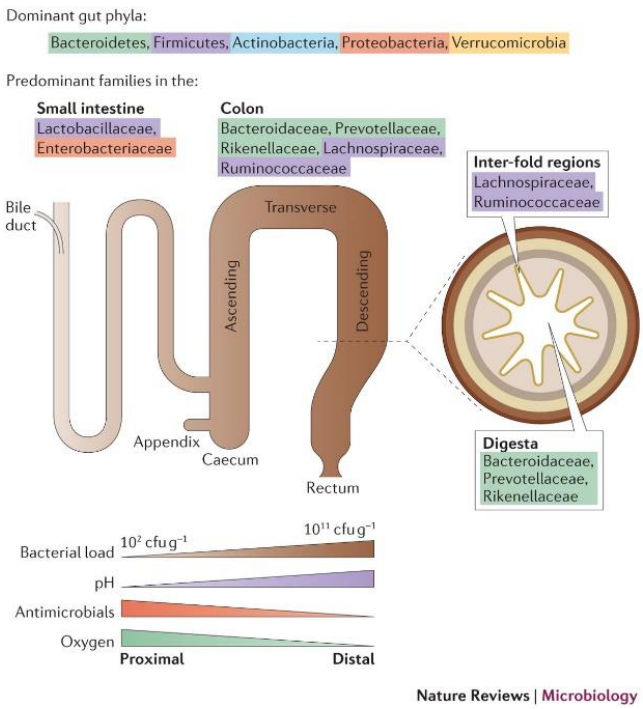


Figure 1: Biogeographical representation of the predominant families in the small and large intestines, based on their diversity and gradients of pH, antimicrobial peptides, and oxygen levels from Donaldson et al.[6].

The densely populated colon ecosystem is comprised of a diverse array of bacterial species, with four main phyla dominating: Firmicutes and Bacteroidetes, which account for approximately 80% of the colonic microbiota, along with Actinobacteria and Proteobacteria, and to a lesser extent Verrucomicrobia (Figure 2)[8]. Over 1,000 species have been identified in studies across individuals, with each individual harbouring at least 150 different species. This highlights that while inter-individual variability exists, there is a core microbiota shared among the population[4]. The most commonly dominant genera among Western populations are *Bacteroides, Eubacterium, Faecalibacterium, Alistipes, Ruminococcus, Clostridium, Roseburia* and *Blautia*, as identified in various population cohorts[9,10]. Compositional clusters can be observed in the human gut microbiota across populations, with two genera*, Prevotella* and *Bacteroides*, driving significant differences between Western and non-Western microbiota compositions[11–13].

To enable comparison between individuals and classify the human gut microbiota into different community types based on the abundance of their dominant genus, a large study comparing gut microbiota composition data from Western populations showed that the gut composition could be split into distinct community types. These community types, referred to as enterotypes, are defined as more or less well-delineated communities of microorganisms forming a specific ecosystem[14]. Initially, three enterotypes were described in a worldwide study including subjects from North America, Europe, and Japan[14]. The *Bacteroides* enterotype (Bact1) is defined by a high abundance of the *Bacteroides* genus, the *Prevotella* enterotype (Prev) by a high abundance of *Prevotella*, and the *Ruminococcus* enterotype (Rum) by a high abundance of the *Ruminococcaceae* family[9]. More recently, another enterotype was defined which split the *Bacteroides* enterotype into *Bacteroides* 1 and 2 (Bact1 and Bact2). Bact2 is characterised by a low microbial load as well as lower relative abundance of the butyrate-producing bacterium *Faecalibacterium* [15,16]. The Bact2 enterotype has been associated with a number of diseases such as inflammatory bowel disease (IBD)[15], obesity[17] or depression[18]. In the Flemish population based on the Flemish gut flora project (FGFP)[9], most individuals belong to Bact1 and Rum enterotypes (approx. 70%, Figure 2A). Prev enterotype has been associated with vegetarianism and more fibre intake [14,19], conversely, *Bacteroides* enterotypes have been associated with protein and animal fat-rich diets (Figure 2B).

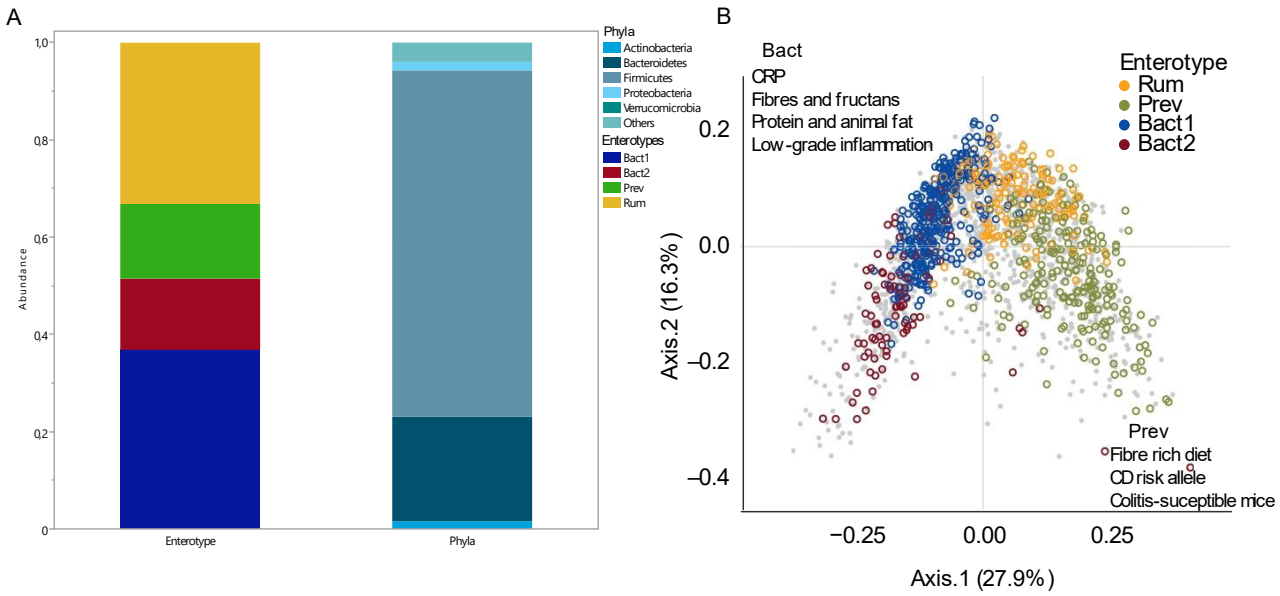


Figure 2. A. Proportion in the FGFP[9] (n =1,106) of each enterotype in the dataset and the median phylum abundance. B. PCoA of a Western cohort where the four defined enterotypes are shown. For Bact and Prev enterotypes, a summary of the diseases and dietary constituents that have been associated with Prev, and Bact1&2 (Bact) enterotypes (Figure adapted from[20] and [17]).

In addition to the enterotype concept, another key pattern has been observed upon comparing the microbiome compositions of industrialised and non-industrialised populations. The phenomenon known as VANISH (Volatile and/or Associated Negatively with Industrialised Societies of Humans) taxa, which encompass members of the *Prevotellaceae*, *Spirochaetaceae*, and *Succinivibrionaceae* families, show a consistently diminished presence or abundance in urbanised cohort populations.[11,13]. The gradually increasing differences in lifestyle factors, such as diet, antibiotic use, and sanitation levels, have altered the industrialised microbiota over time[11]. Although the cause-consequence relationship between modern diseases such as obesity and the loss of these VANISH taxa is not yet well understood, the "disappearing microbiota hypothesis" suggests that this loss may be the direct cause of these diseases.[21,22]. If proven correct, restoring the gut microbiota by incorporating or stimulating these VANISH taxa may be a means of mitigating these diseases. Nevertheless, a deeper understanding of the dynamics of the gut ecosystem, its connection to the host, and its relationship with the immune system is required.

Bacteria are the most extensively studied component of the gut microbiota, but the gut also contains a number of other inhabitants, including archaea[23], viruses[24] and eukaryotes[25]. Recent advancements in the sequencing technology have revealed that the human gut

archeome is primarily composed of methanogens from the *Methanobrevibacter* and *Methanosphaera* genera. However, the interaction between these archaea and the host and bacteriome remains largely unknown[26]. The gut virome comprises approximately 54,000 viral species and an estimated 10^{12} virus-like particles per person. This virome includes both eukaryotic viruses (which infect the host) and prokaryotic viruses (which infect bacteria), with the most abundant belonging to the *Ackermannviridae, Chaseviridae, Herelleviridae, Demerecviridae, Drexlerviridae* and *Autographiviridae* families (dsDNA viruses). Viruses play crucial roles in the gut ecosystem by significantly affecting the composition and evolution of the bacteriome and by mediating crucial interactions with the host immune system. These interactions are necessary for the development and functioning of the host immune system[27].

Furthermore, the gut microbiota also contains eukaryotic organisms, including metazoan parasites (e.g. nematodes and helminths), fungi (e.g. filamentous fungi and yeasts), and protozoa (i.e. single celled eukaryotes such as *Blastocystis)*[25]. Historically, these organisms have been primarily studied in the context of their parasitic or pathogenic relationships with the host. However, their absence has also been associated to disease, as in the case of *Blastocystis* in patients with IBD[28]. Future studies on the microbiome should therefore encompass interkingdom interactions and a comprehensive analysis of community composition rather than focusing only on bacteria.

Studying the gut microbiota composition: culture-independent vs culture-dependent methods

Cultivation methods have been the traditional approach for studying microorganisms, but were primarily limited to quantification of growth, using colony forming units (CFUs) on solid media or turbidity in liquid media. Additionally, phenotypic information such as the ability to perform oxidative or catalytic processes, can be obtained through biochemical tests and gram-staining, which allows differentiation between gram-negative and gram-positive cells[29]. These assays have been used to study the diversity of the gut microbiota, and initially, it was believed that it was mainly dominated by *Enterobacteriaceae* as well as *Bifidobacterium* and *Bacteroides* spp[8]. These taxa are less challenging to cultivate and also fast-growing and tolerant to oxygen. The advent of anaerobic techniques, such as Hungate tubes

in the 1960s and the use of anaerobic chambers, has enabled a deeper understanding of gut composition by enabling a wider range of culturable species in the lab.

However, advancements in culture-independent sequencing technologies rapidly circumvented the limitations posed by cultivation methods, providing a more comprehensive picture of microbial diversity. The use of 16S rRNA gene sequencing, a molecular target, allows for the classification of bacterial species based on their 16S rRNA gene sequence similarity, whereas earlier bacterial taxonomy was established through biochemical or morphological tests. Studies of healthy individuals and clinical cohorts have contributed to a broadened understanding of the taxonomic diversity present in the human gut[30,31]. However, it must be noted that 16S rRNA gene amplicon sequencing is mostly limited to genus-level resolution and lacks the discriminatory power to identify putative targets at sufficient taxonomic depth, as well as the ability to accurately unveil their metabolic potential. Shotgun sequencing metagenomics, on the other hand, provides strain-level resolution and enables the identification of different bacterial strains and genes[32]. The use of recent long read technologies such as Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) can also aid in sequencing microbiomes and complete bacterial genomes obtained from samples[33] (Figure 3). Nevertheless, it is important to keep in mind that the results of sequencing analyses depend on the sequencing depth and taxonomic coverage, and rare taxa may not always be detected in the sample. Additionally, biases occurring during the DNA extraction and the PCR procedure (for amplicon-based sequencing), could also have a significant impact on community profiling, as they can result in an inaccurate representation of the microbial communities being studied[34].

The use of a combined approach that incorporates both culture-dependent and culture-independent methods has demonstrated that a more comprehensive understanding of gut composition can be obtained[35]. Culturing enables a creation of culture collections and a production of high-quality genomes through whole genome sequencing (WGS). Using stool samples, bacteria can be isolated and identified through various methods: whole genome sequencing, Sanger sequencing (for 16S rRNA gene sequencing) or matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) based on their peptide mass fingerprint (Figure 3). While sequencing technologies provide valuable information on the potential functions of microbes, these putative functions can only be

validated through in vitro studies using isolated strains. Additionally, the sequencing information only relies on existing databases, and new functions cannot be discovered using only sequencing. Thus, despite its limitations, culturing remains an essential tool in the study of the gut microbiota. Culturing is the only technique that allows to gain insights into community interactions beyond predictions, by studying in vitro microbe-microbe interactions in co-cultures or mixed communities and therefore study the impact of these interactions on growth and metabolism. The scientific challenge and potential for biotherapeutic and biotechnological applications make the study of gut microbiota through cultivation an important area of research.

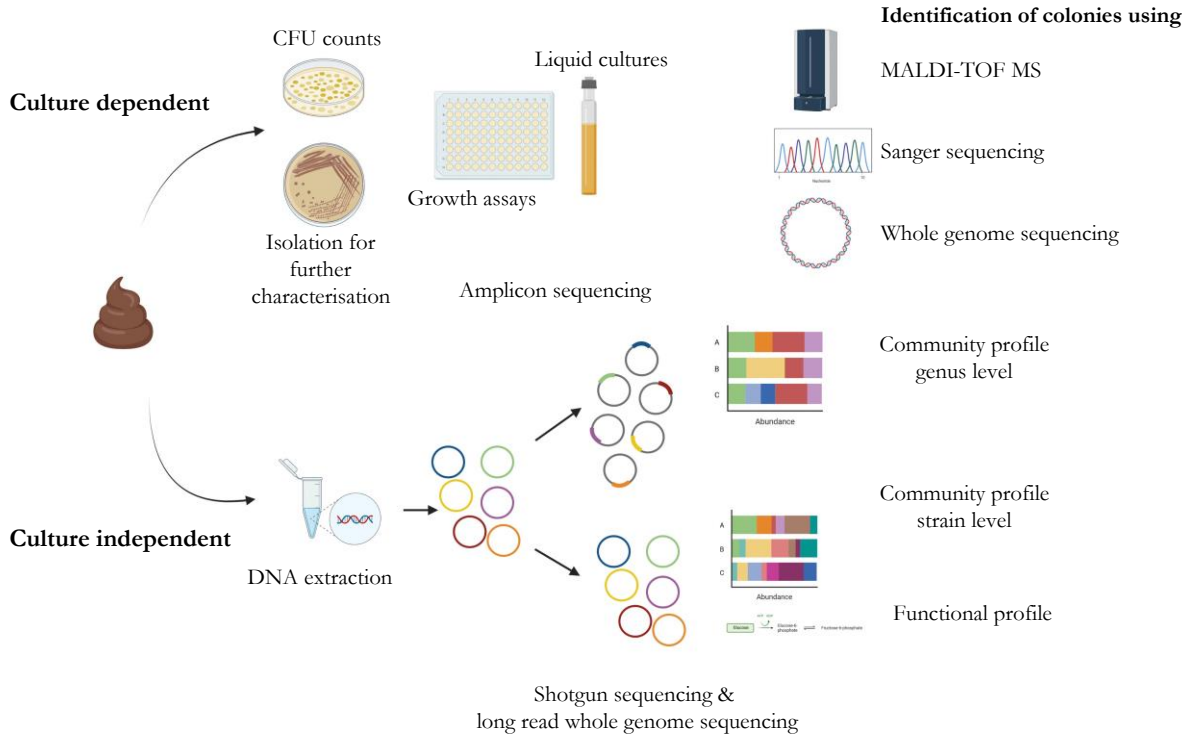


Figure 3. An overview of culture-dependent and independent methods. While culture-dependent methods rely on bacterial growth and isolation to enable in depth characterization and understanding of their metabolism as well as microbe-microbe interactions. On the other hand, culture independent methods enable a blueprint of the gut bacterial species and potential metabolism if whole genomes are sequenced (shotgun sequencing & long read sequencing). Combining both approaches in parallel shows to be a good complementary approach to study community composition. Image made with Biorender.

Microbial interactions and metabolism in the human gut microbiota

The colon ecosystem is composed of diverse taxa, each having a distinct role in the gut ecosystem. Some species specialise in aiding the host in the digestion of complex carbohydrates and proteins[36], such as cellulose, that human enzymes cannot process[37], serving as primary degraders. Others, acting as secondary degraders, utilise the metabolic end-products of the primary degraders through a process referred to as metabolic cross-feeding. For instance, *Ruminococcus bromii* degrades resistant starch thereby promoting the growth of species such as *Agathobacter rectalis* that have limited ability to degrade this substrate[38]. Additionally, butyrate producers, such as *Faecalibacterium prausnitzii*, require intermediate by-products, primarily acetate, for butyrate production, which can be acquired from other members of the community through cross-feeding[39]. These cross-feeding interactions are part of distinct trophic levels, wherein specific species within the community consume nutrients to produce metabolites. These metabolites subsequently serve as a resource for other community members (e.g., the degradation of polysaccharides to yield simple sugars or the utilization of lactate to produce butyrate). Although cross-feeding represents a positive form of interaction, negative interactions, such as competition for shared resources, can also arise among community members[40]. Furthermore, a stable microbiota is characterised by functional redundancy, whereby a number of species have similar functions, enabling compensation for changes in the composition without dramatically altering overall functioning of the ecosystem[41].

The human gut microbiota is involved in a range of metabolic processes. One of the best understood functions is the conversion of carbohydrates from undigested fibres into short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. The gut microbiota also contributes to the synthesis of important compounds, including vitamins. For example, members of the *Bacteroides* genus can synthesise vitamins K and B, which are crucial for overall health[42,43]. Additionally, the gut microbiota can detoxify antinutrients present in the diet, such as phytates (by *Bifidobacterium pseudocatenulatum*[44]), or tannins[45], breaking down harmful compounds and making nutrients, such as minerals, more accessible to the host. Another significant process, the conversion of bile acids, is also performed by some members of the gut microbiota. For instance, *Bacteroides intestinalis* is able to convert primary bile acids into secondary acids[46].

The degradation of undigested carbohydrates and proteins leads to the generation of metabolic end-products, such as hydrogen, carbon dioxide, methane, SCFAs, and free amino acids[47]. These end-products are not only utilised by the microbiota but also benefit the host. For instance, some SCFAs, such as butyrate, have anti-inflammatory properties and may play a role in defence against pathogens by activating the host's immune system (e.g. activation of Nuclear Factor-$\varkappa$B)[48]. A summary of the most studied microbial metabolites and their role in health can be found in Table 1. The major pathways leading to the biosynthesis of these major metabolites from carbohydrates are shown in Figure 4.
The measurement of microbial metabolites, referred to as metabolomics, is a valuable approach in microbiology as it directly reflects the biochemical activity of microbes or microbial communities. This is achieved through metabolomic profiling, which can be executed using either targeted or untargeted methods. Targeted methods monitor specific known metabolites, while untargeted methods encompass a global profiling approach[49].

Table 1. A summary of the most studied microbial metabolites in the gut microbiota with their synthesis pathways as well as examples of producers and health benefits.

MICROBIAL METABOLITE	PRODUCER (EXAMPLE)	HEALTH BENEFITS (EXAMPLES)
ACETATE	*Bacteroides* spp.[50]	↓ gut permeability ↑ immune surveillance (via IgA) Intermediate to produce other metabolites such as butyrate.[50]
BUTYRATE	*Faecalibacterium* spp.[51]	Anti-inflammatory[50]
FORMATE	*Ruminococcus bromii*[50]	Intermediate to produce other metabolites such as acetate. Important in many host biosynthetic pathways (ex. T cell proliferation)[52]
LACTATE	*Bifidobacterium* spp.[50]	Intermediate to produce other metabolites such as butyrate.[50]
PROPIONATE	*Coprococcus catus*[53]	Anti-inflammatory, lowers lipogenesis[50]
SUCCINATE	*Prevotella copri*[54]	Intermediate to produce other metabolites such as propionate.[50]

To detect, characterise and quantify microbial metabolites, various analytical platforms, including gas chromatography (GC), liquid chromatography (LC), high-performance LC (HPLC), ultra-performance LC (UPLC) but also Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) can be employed. Additionally, mass-spectrometry (MS) metabolomics is largely used for untargeted metabolome analysis to identify and quantify the composition of complex mixtures[49].

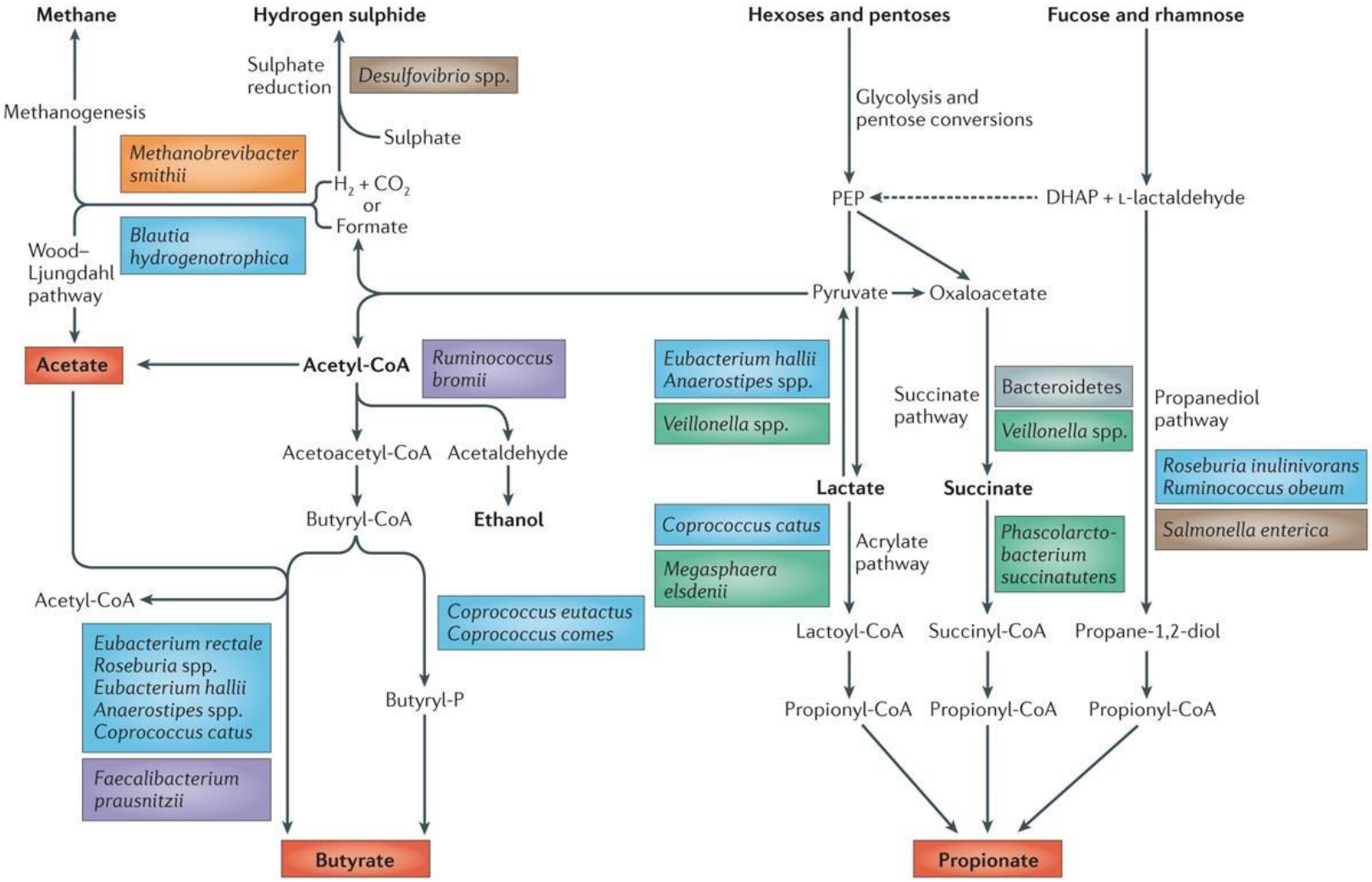


Figure 4. The main pathways leading to the biosynthesis of the major metabolites from carbohydrates, mainly SCFAs and gases (CH_4, H_2S) from Louis et al[55].

Human gut microbiota role in health and disease

The gut microbiota diverse and interactive structure confers tolerance to potential pathogenic invasions and environmental changes[56,57]. This attribute is commonly referred to as resilience and is defined as the amount of stress or perturbation that a microbial ecosystem can withstand before changing into a different equilibrium state[56]. A key aspect of the resilience of the community, is to be functionally redundant and able to be restored after a perturbation, due to its diverse set of microbes (Figure 5A). This resilient microbiota is not necessarily composed of a set of core microbes shared by a population but rather of a functional core[56,58], which is formed during childhood[56], and evolves overtime as it is significantly affected by diet[59]. During early life, the diversity of the gut microbiota is lower and dominated by more facultative or oxygen-tolerant taxa such as species belonging to the *Streptococcus* and *Bifidobacterium* genera, but as it matures it becomes more dominated by *Bacteroides* spp. and Firmicutes including butyrate producers, more closely resembling an adult microbiota[60].

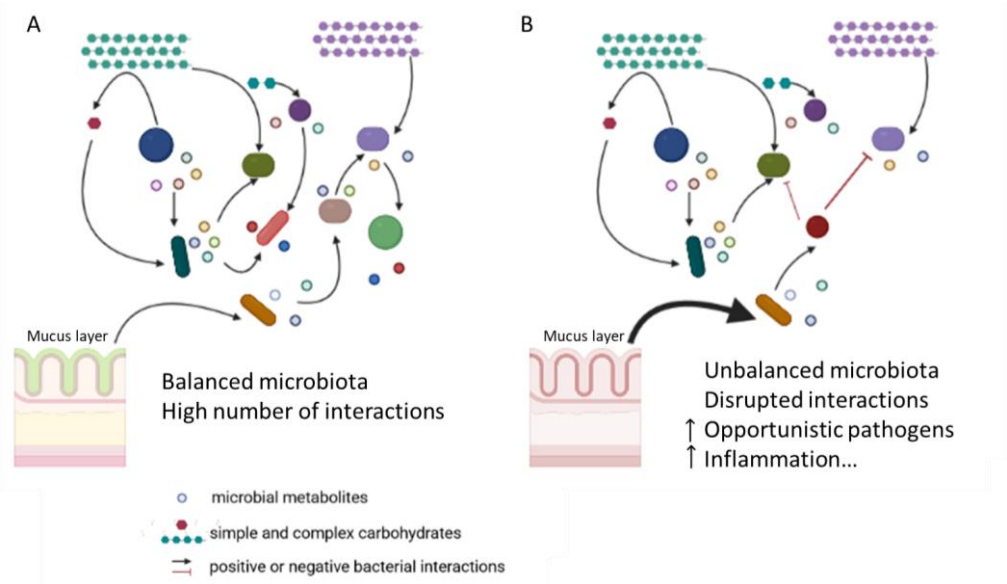


Figure 5. A. The human gut microbiota is a complex and interactive ecosystem that depends on primary consumers breaking down large molecules such as undigested fibres and host-derived glycans (i.e., mucin). The proper functioning of this ecosystem requires functional redundancy and high levels of interaction among the community members. B. When the community is disrupted by factors such as pathogens or antibiotics, the mucus barrier can be weakened and further degraded by the gut microbiota, leading to an increase in inflammation that is often already present during dysbiosis. During dysbiosis, the interactions within the ecosystem become disturbed, leading to the absence of certain taxa and the loss of equilibrium in the gut microbiome. Image made with Biorender.

However, when environmental perturbation is exceptionally high, the microbiota moves towards an imbalanced state known as dysbiosis. This reflects an overall imbalance of gut ecosystem homeostasis manifested by reduced taxonomic diversity, alteration or loss of important metabolic functions, and overgrowth of pathogenic or opportunistic bacteria[61], all of which increase the risk of chronic inflammatory state (Figure 5B) [56,62]. Diseases associated with dysbiosis such as IBD are characterised by inflammation of the mucosal layer[63,64], as well as other diseases such as colorectal cancer[65] and diabetes (type 1 or type 2 diabetes[66]). Dysbiosis has also been linked to non-GI tract-related disorders such as neurodegenerative disorders like multiple sclerosis[67], Alzheimer's[68] and Parkinson's diseases[69]. The main changes observed in dysbiosis include variations in the Bacteroidetes/Firmicutes ratio and reduced diversity in the most abundant taxa compared to healthy individuals[7,66].Due to the changes in bacterial composition, recently, the aforementioned Bact2 enterotype has been proposed as an inflammation-associated dysbiotic state[15]. As genetic factors and environmental triggers are also associated with these pathologies, further insight is needed as the relationship between these changes and disease remains unclear[1,20]. However, the loss of microbiota equilibrium has been shown to have consequences for the host including disruption of the gut barrier and imbalance of the host immune and metabolic systems. Indeed, dysbiosis has been shown to lower immunity during infections, further emphasizing the importance of a resilient microbiota in defending against pathogenic organisms[65]. Additionally, dysbiosis affects the host metabolic system, particularly glucose and lipid metabolism, metabolisms that are mediated by the gut microbiota via bile acid composition or the production of SCFAs from dietary fibres[70] and therefore disturbed during dysbiosis.

Restoring gut health

The human microbiota is receiving increasing attention as a target for diagnosis, therapy, and disease prevention, given its crucial role in health and disease. A prime example of the therapeutic potential of the colon microbiota is the use of faecal microbiota transplantation (FMT), which has been successful in treating recurrent *Clostridioides difficile* infections (CDI) and IBD by shifting the microbiota composition of the patient to a healthier state that more closely resembles that of the non-inflamed donor[71,72] (Figure 6). However, some side

effects, such as abdominal discomfort, fever, diarrhoea, vomiting, and constipation have been reported (reviewed in[73]), and the effectiveness of the FMT relies on fresh samples from specific effective donors[73], which requires careful screening to avoid pathogen transmission[74]. Additionally, FMTs lack standardization with respect to the administration, selection of donors, and even their preparation, thereby making comparisons between studies challenging[75].

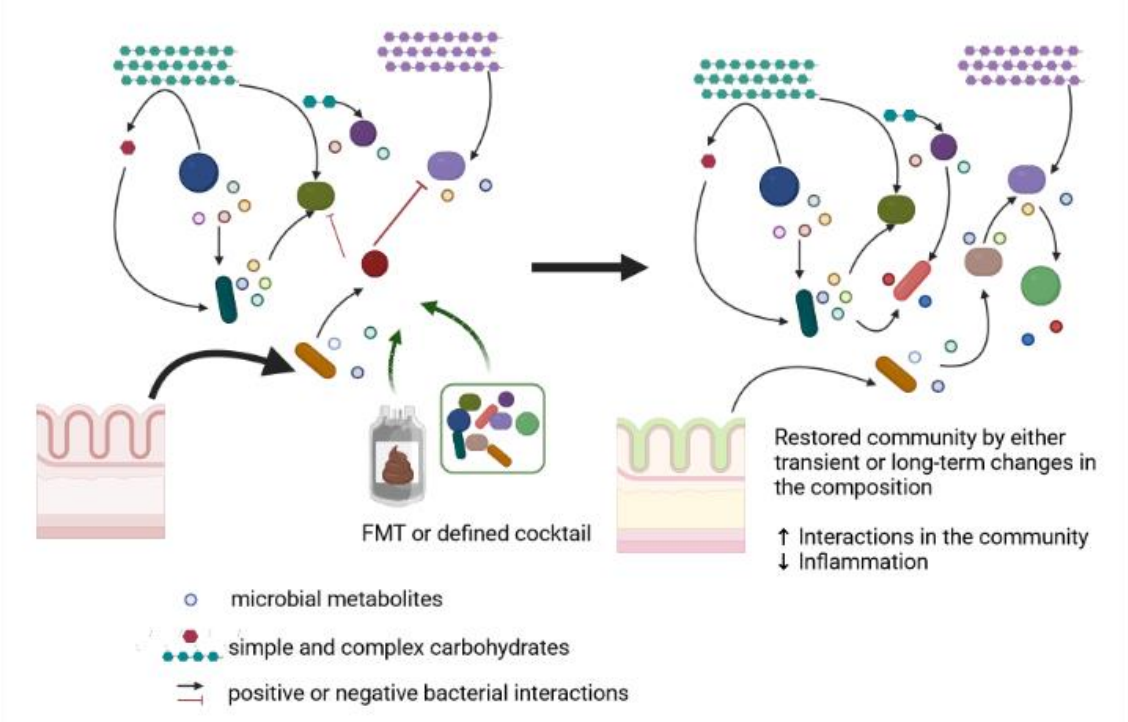


Figure 6. In the event of dysbiosis, it becomes necessary to restore the gut microbiota to its previous state. This can be accomplished through either an FMT using a full microbiota from a healthy donor, or by administering a defined and selected cocktail of microorganisms capable of restoring the disrupted ecosystem. Either method has the potential to bring the gut functions and interactions back to its previous, more resilient state. Image made with Biorender.

For these reasons, the design and use of defined inocula provides a more sustainable path for further therapeutic interventions at the microbiota level[76]. While this has attracted a significant attention from the probiotic industry, in adults for instance, most of commercially available probiotic products were initially largely selected based on technological properties rather than health benefit using either a narrow spectrum of easily culturable food-grade organisms such as lactic acid bacteria or not ecologically fit bifidobacteria[77] but have a long history of use[76]. Although health benefits of current probiotics may be independent to gut microbiome modulation, there is an interest

to identify strains for new biotherapeutics[78]. Therefore, there is an increasing interest in using colonic, more recently characterised gut-derived strictly anaerobic species such as *Faecalibacterium prausnitzii*[79,80], *Butyricicoccus pullicaecorum*[81] and *Akkermansia muciniphila*[82] as next-generation probiotics, due to their potential for producing health-supporting metabolites such as butyrate, an anti-inflammatory SCFA[83]. Additionally, there is growing evidence suggesting that multi-species probiotic formulations and synthetic communities are more efficient than single strains in restoring gut ecosystem homeostasis as shown through a comparison of clinical trials that treated CDI with single-species or multi-species probiotics[84] (Figure 6).

Such multispecies approach opens up several research questions to deal with the complexity of design, production and management of microbial cocktails, i.e., the mechanistic understanding of the interactions that shape the functional stability and performance of the multi-strain consortium, and how this microbial interplay influences the biotherapeutic potential of the preparation towards restoration of gut diversity. Probably the most well-known example of these interactions is metabolic cross-feeding[85] . However, many other intricate interactions in the gut ecosystem are not well understood, as only few studies have dissected microbiota functioning as such in in vitro or in vivo settings[86,87].

Defining gut communities towards designing next generation therapeutics

To date, bacterial cocktails are usually administered as a mix of individual members instead of a cocultured community mostly for practical reasons[88,89]. However, the selection of such cocktail should be carefully done. Assembly of novel bacterial cocktails to target a dysbiotic gut ecosystem strongly relies on the identification of microbiome members and/or functions that are specifically related to disease onset and progression. The accuracy of these analyses is heavily dependent on the experimental design, the host population structure, the selection of the non-diseased control group and, a sufficiently large sample size to provide the power to detect differences between conditions[9,90]. Accurate identification of microbial targets is dependent on omics-driven analyses.

However, profiling alone does not predict the bacterial interactions or the host-bacteria interactions that are occurring[2,91]. For this purpose, network-based and correlation analyses

are a powerful tool to identify putative probiotic cocktails, either by detecting bacterial communities that offer a benefit to the host or by identifying organisms that can outcompete pathogenic species. In such models, nodes typically represent taxonomic groups and edges statistical or metabolic dependencies. Finally, metabolic information can be used for genome-scale metabolic modelling (GSMM) and flux balance analysis[92,93]. These tools predict and provide the cross-feeding and competition mechanisms necessary, although not sufficient in order to design a multi-strain formulation[92,94,95]. They also allow for designing the optimal bacterial community for a desired metabolite, for example butyrate production[96], and predict the growth medium for commensal species[97]. Nevertheless, these approximations rely on the quality of gene functional assignments and often depend on manual refinement of genome annotations[94,98]. The GSMM tools used to create putative metabolic networks and predict interactions can be applied to understand potential growth needs and can help to define a common growth medium to allow co-establishment of all selected consortium members[97] (Figure 7). An alternative approach to study interactions is network/correlation analysis, in which similarities in abundance profiles of organisms is used as a proxy for their physical or metabolic interaction[2,91]. In the derived networks nodes typically represent taxonomic groups and the edges statistical or metabolic dependencies. Different approximations exist to infer species interaction networks, and their performance has been widely reviewed [91,99]. However, methods that can cope with the compositionality, the sparseness and the high dimensionality of metagenomic data appear to show the best performance [100,101]. Once the network is estimated, different clustering algorithms [99] can be used to detect putative bacterial communities. Moreover, methods such as Bayesian networks can integrate the information from clinical metadata into the network inference [102,103] and select the specific set of nodes that affect e.g. production of SCFA or immune responses [103,104] Such patterns can also be represented using heatmaps (Figure 7).

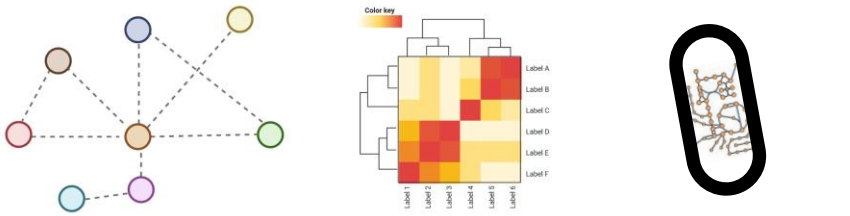


Figure 7. Representation methods to show interactions (network based analysis and co-occurrence patterns) and in silico metabolic potential of bacterial species using GSMMs. Image made with Biorender.

A recent shotgun metagenomics study identified about 2,000 uncultured bacterial species, highlighting the challenges of in vitro work starting from the isolation of selected strains[105,106]. However, understanding mixture behaviour remains a necessary step in the design process, even though predictions generally provide incomplete insights[107]. It is recommended to test 'universal' growth media in parallel with media supplemented with additional growth factors in monocultures. To infer cross-feeding interactions and identify specific metabolites as growth-promoting factors, the use of spent media (i.e. media harvested from cultured cells) to culture other members of the consortium can be used[108] (Figure 8A). Once the medium is defined, different in vitro configurations can be applied to test the functioning and behaviour of the synthetic community.

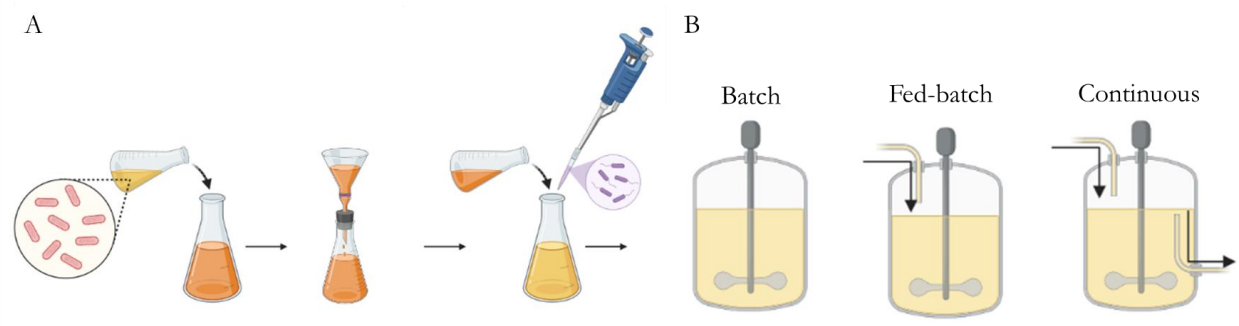


Figure 8. A. An example of an in vitro experiment to infer interactions, by using spent medium to study cross-feeding potential. The medium of species 1 is filtered and used as growth medium for species 2. B. There are three distinct culture settings: batch, fed-batch, and continuous. In batch culture, the medium is not replenished, resulting in a closed system. In contrast, fed-batch culture involves the addition of fresh medium to renew nutrients. Finally, continuous culture involves the continual addition of fresh medium and removal of medium containing cells and metabolites. Image made with Biorender.

While traditional batch cultures are limited by the carbon source availability and/or the potential accumulation of toxic metabolites, fed-batch cultures allow some renewal of substrate enabling a higher cell density and preventing substrate inhibition. Continuous fermentations in chemostats allow for a constant addition of substrate and removal of medium and cell biomass, imposing kinetic conditions and growth rates limitations on the consortium (Figure 8B). Additionally, a pH-controlled set-up avoids undesired changes or even elimination of some members from the community[109].

To make these in vitro models physiologically relevant, complex fermentation configurations have been developed to mimic colonic systems and reproduce the kinetic characteristics of the major compartments of the intestinal tract[110]. These systems have been used to study the impact of certain substrates, such as inulin[111], as well as to investigate the effect of plant-derived compounds on the gut-like ecosystem[112]. More advanced in vitro colon simulators add another layer of complexity by including host parameters such as mucus layer and human cell lines[113]. The incorporation of mammalian cell components such as intestinal organoids, 2D cell models and transwell cultures allows the study of host responses of colonocytes and immune cells to microbial consortia and their metabolic outputs[114–116]. The host-microbe interactions envisaged here range from immune responses, exchange of metabolites to adhesion properties of bacterial cells.

Furthermore, temporal dynamics of the in vitro behaviour and stability of the consortium can be assessed using a combination of 16S rRNA gene sequencing (for taxonomic composition), absolute quantification through cell counting[16], and different modelling approximations[86]. Metabolic and transcriptomic profiles can be acquired to assess phenotyping responses of the community compared to in silico predictions, but more importantly to functionally validate the consortium[97,98].

A better understanding, and ultimately, a prediction of gut species interactions is thus truly necessary to rationalise the future design of synthetic microbial biotherapeutics. The use of ecological and physiological principles in the development of probiotic cocktails would improve their effectiveness in restoring gut diversity and function to treat gastrointestinal related disorders. In order to design next generation biotherapeutics, both in silico selection of bacterial candidates as well as predictions of their function and interactions coupled with

in vitro validation are crucial. Indeed, the identification of novel bacterial cocktails to target a dysbiotic gut ecosystem strongly relies on the identification of microbiome members and/or functions that are specifically related to disease onset and progression.

Objectives

The gut microbiota is crucial in human health, and dysbiosis has been associated with many diseases. While culture-independent surveys have provided comprehensive cross-sectional views of the human gut microbiota composition, an in-depth understanding of how its members function together as a community is still lacking. The application of culture-dependent approaches is required to address the knowledge gap on how gut communities function as an ecosystem and restore a functionally balanced state in disease settings. As the gut is a highly complex ecosystem comprising hundreds of species, unravelling interactions at community level is very challenging. To this end, the use of simplified communities could be a first step.

Conceptually, the study of simplified communities can be achieved in two ways: i) using a bottom-up approach, where isolated strains are assembled into a community, or ii) using a top-down approach, where a complex community (e.g., a faecal sample), is reduced into a simpler community by culture-based enrichment. The bottom-up approach not only enables a careful selection of bacterial members considering their prevalence in the population as well as their functionally interesting properties, but also allows defining the degree of community complexity. However, assembling strains from different donors could lead to unwanted exclusion and competition in the assembled community. On the other hand, the top-down approach may yield more ecologically stable communities of members with a common donor history, but the functional breadth of the resulting community is more unpredictable. Both approaches are complementary and can provide new insights into how phylogenetically and functionally diverse bacteria coexist in the gut environment and interact using simpler communities.

This thesis aims to use simplified communities to understand and design bacterial interactions between selected gut microbiota members. The first objective of this thesis is to build a culture collection comprising diverse bacterial members of the gut microbiota (**Chapter 2**). This step is crucial to assemble defined communities using a bottom-up approach. This will be achieved through an anaerobic isolation campaign using faecal samples of healthy human volunteers that are cryopreserved under different conditions.

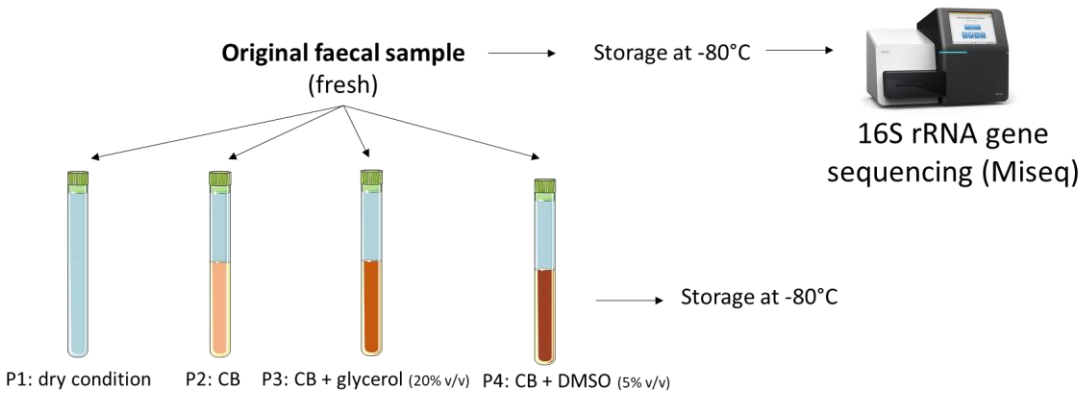
The second objective of this thesis is to select core members of the microbiota and gather information on their metabolic capabilities to form two functional core consortia based on the *Prevotella* and *Bacteroides* 1 enterotypes (**Chapter 3**). This step will be performed in silico using a previously generated 16S rRNA gene sequencing data set from 1,106 subjects participating in the Flemish Gut Flora Project. Then, a third aim is to successfully assemble these communities in vitro and to learn more on the necessary steps to build defined communities (**Chapter 4**).

Finally, in order to complement the bottom-up approach, a top-down approach will also be explored. A fourth aim of this thesis is therefore to assess the effect of different substrates on resulting community compositions and interactions through substrate-driven enrichments instead of predetermined selection. First, by testing similar donors (**Chapter 5**) but also using diverse donors, by comparing one *Bacteroides* 1 and one *Prevotella* enterotype sample (**Chapter 6**). This will allow us to evaluate how variations in substrates can affect community dynamics.

Chapter II
Effect of cryopreservation medium conditions on growth and isolation of gut anaerobes from human faecal samples

This chapter has been published in:

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Original faecal sample
(fresh)
Storage at -80°C
16S rRNA gene
sequencing (Miseq)
Storage at -80°C
P1: dry condition
P2: CB
P3: CB + glycerol (20% v/v)
P4: CB + DMSO (5% v/v)

Abstract

Novel strategies for anaerobic bacterial isolations from human faecal samples and various initiatives to generate culture collections of gut-derived bacteria have instigated considerable interest for the development of novel microbiota-based treatments. Early in the process of building a culture collection, optimal faecal sample preservation is essential to safeguard the viability of the broadest taxonomic diversity range possible. In contrast to the much more established faecal storage conditions for meta-omics applications, the impact of stool sample preservation conditions on bacterial growth recovery and isolation remains largely unexplored.

In this study, aliquoted faecal samples from eleven healthy human volunteers selected based on a range of physicochemical and microbiological gradients were cryopreserved at -80°C either without addition of any medium (dry condition) or in different Cary-Blair medium conditions with or without a cryoprotectant, i.e., 20% (v/v) glycerol or 5% (v/v) DMSO. Faecal aliquots were subjected to bulk 16S rRNA gene sequencing as well as dilution plating on modified Gifu Anaerobic Medium after preservation for culturable fraction profiling and generation of bacterial culture collections.

Analyses of compositional variation showed that cryopreservation medium conditions affected quantitative recovery but not the overall community composition of cultured fractions. Post-preservation sample dilution and richness of the uncultured source samples were the major drivers of the cultured fraction richness at genus level. However, preservation conditions differentially affected recovery of specific genera. Presence-absence analysis indicated that twenty-two of the 45 most abundant common genera (>0.01% abundance, dilution 10^{-4}) were recovered in cultured fractions from all preservation conditions, while nine genera were only detected in fractions from a single preservation condition. Overall, the highest number of common genera (i.e. 35/45) in cultured fractions were recovered from sample aliquots preserved without medium and in the presence of Cary-Blair medium containing 5% (v/v) DMSO. Also, in the culture collection generated from the cultured fractions, these two preservation conditions yielded the highest species richness (72 and 66, respectively).

Our results demonstrate that preservation methods partly determine richness and taxonomic diversity of gut anaerobes recovered from faecal samples. Complementing the current standard practice of cryopreserving stool samples in dry conditions with other preservation conditions, such as Cary-Blair medium with DMSO, could increase the species diversity of gut-associated culture collections.

Introduction

While metagenomic profiling has become the standard approach to study gut microbial ecosystems, awareness is growing that parallel efforts in cultivation-based approaches are indispensable to fully comprehend the functional capacities of its members and their interactions[117]. The availability of pure microbial cultures isolated from human gut samples is one of the key elements in the biological validation of host-microbiota associations inferred from metagenomics-based readouts[118]. In addition, building culture collections of gut anaerobes can also promote bioprospection of strains with novel functionalities and the description of novel species[29,119,120] and, ultimately, lead to the development of new biotherapeutics[1].

From sample collection to strain cataloguing, the generation of microbial culture collections can be a long and complex process. For gut-derived collections, safeguarding anaerobic conditions during faecal sampling and processing to minimise losses in viability and culturability of oxygen-sensitive organisms poses an additional challenge. Although immediate processing after sampling is highly preferred for microbial isolation purposes, this is not always possible due to complex logistics and sample sizes of most human cohort studies. Therefore, intermediate sample preservation is often a necessity during large-scale sampling campaigns, given that cultivation is a lengthy process and sample throughput is usually limited. Many different methods for long-time cryopreservation of faecal specimens have been described, all of which usually involve freezing at -20°C or -80°C with or without addition of cryoprotectants (e.g. glycerol or dimethyl sulfoxide - DMSO) and/or protective buffers[121–123.] In the gut microbiome field, evaluation of these methods has mostly focused on the integrity of nucleic acids[124,125] rather than on the recovery of culturable microbes. Yet, DNA-based profiling and microbial isolation are very different processes. When comparing sample preservation conditions, it is not only necessary to investigate how community composition and diversity are potentially impacted, but also to assess if different conditions ensure viability and would promote growth and isolation of taxonomically different strain sets.

To date, reports on the recovery of (living) faecal communities or specific commensal gut species are very limited. Alternatively, important insights can be obtained from studies on the post-preservation recovery of artificial microbial communities and fermented faecal

samples. Kerckhof and colleagues reported that overall community compositions of faecal fermentation samples after preservation in DMSO (5% v/v), DMSO with trehalose and tryptic soy (5%, 1%, 0.3% v/v), or without protective agents were not impacted by any condition[126]. The authors pointed out that 15% of operational taxonomy units (OTUs) were not recovered in at least one condition, however, these OTUs were recovered better after preservation with cryoprotective agents. Similarly, Bircher and colleagues showed that butyrate producers recovered well from artificial microbiota cryopreserved in a medium containing glycerol and inulin (15%, 5% v/v)[127]. These studies reinforce the important role of cryoprotective media in maintaining community integrity and recovery of taxa in complex communities. Still, taxon-specific recovery differences are likely to occur under any given cryopreservation condition[126]. In another study Bircher and colleagues confirmed species-specific differences in the viability of obligately anaerobic gut isolates following cryopreservation at -80°C in sucrose and inulin (5% v/v each), sucrose, inulin and glycerol (5%, 5% and 15% v/v), lyophilisation, or storage at 4°C. Overall, species best recovered from cryopreservation in the sucrose, inulin and glycerol combination, while lyophilisation was more detrimental[128].

While in previous studies different temperatures were tested on the recovery of communities and isolates, the impact of medium was not. In the current study, we compared four preservation conditions that differed in the addition of Cary Blair (CB) medium and/or the choice of cryoprotectant added while keeping the storage temperature constant at -80°C. CB is an isotonic nutrient-free transport medium for faecal samples characterised by a low oxidation-reduction potential and a high pH of 8.4 to protect bacterial cells from acidic shifts [129,130]. We assessed to what extent these different cryopreservation medium conditions impacted microbial richness and culture recovery for a selection of faecal samples collected from 11 healthy human volunteers. In a parallel approach, samples were subjected to both culture-independent 16S rRNA gene community profiling as well as (after preservation) dilution plating for the purpose of culturable fraction profiling and isolation of pure cultures. Culture-based assessments were performed using modified Gifu Anaerobic Medium (mGAM), one of the most commonly used non-selective general media for intestinal anaerobes known to support growth of most predominant gut species[107,119,131]. Comparison of our results across different conditions tested suggests that cryopreservation of faecal sample aliquots under multiple medium conditions guarantees a

larger taxonomic spectrum of isolated species compared to preservation using one single condition, guiding future gut culturomics efforts.

Methods

Ethical compliance

All experimental protocols were approved by the Commissie Medische Ethiek, UZ KU Leuven. Study design complied with all relevant ethical regulations, aligning with the Declaration of Helsinki and in accordance with Belgian privacy laws. All participants gave their informed consent. Ethical approval of the study protocol was obtained (S59288).

Sample collection and preservation

An initial cohort of 51 healthy donors from the KU Leuven community provided the faecal samples used for this study[16]. No inclusion or exclusion criteria were imposed. A limited set of anthropometric metadata was compiled at enrolment, including gender, age, height, and weight. Participants were asked to collect a maximal amount of faeces (single defecation) in a plastic receptacle with lid and to deposit the sample in a labelled non-transparent zip-lock bag at the research facility immediately after defecation. All faecal samples were processed within an hour of the reported time of egestion. Upon collection, fresh faecal samples were homogenised and divided in a series of aliquots for physicochemical analyses, 16S rRNA gene community profiling and comparative analyses of four different conditions. The latter comparison required two aliquots with specific weights. First, 1 gram of fresh faecal sample was stored dry in a cryotube without addition of preservation medium or cryoprotectant (preservation condition P1). In parallel, 3.75 g of fresh sample was resuspended in 15 mL of sterile Cary-Blair transport medium with red phenol indicator (Remel, Lenexa, USA). The resulting suspension was distributed over three different cryotubes each containing four mL of faecal suspension. The first tube was preserved without cryoprotective agent (preservation condition P2), the second was supplemented with 1 mL sterile glycerol (Alfa Aesar, Thermofisher, Germany) to a final concentration of 20% v/v (preservation condition P3) and the third with 0.2 mL DMSO (Sigma Aldrich, USA) to a final concentration of 5% v/v (preservation condition P4; Figure 1).

Faecal samples selection

Out of the initial 51 faecal sample donors, 11 were selected along a gradient of physicochemical (i.e. faecal pH, water activity, and moisture content) and microbiological (i.e. microbial load) parameters (Supplementary Table S1.1). Water activity was measured in duplicate at 37°C using a resistive electrolytic hygrometer (Labmaster, Novasina, Lachen, Switzerland) after manual homogenization of the fresh faecal sample. Faecal pH of fresh samples was measured in triplicate after mechanical homogenization (5 min, 3xg; Stomacher 3500 [Seward Ltd., Worthing, UK]) using a FG2 pH meter coupled to an InLab Solids electrode (Mettler Toledo, Greifensee, Switzerland). Stool moisture content was determined in duplicate on 0.2 g of frozen faecal material (-80°C) as the percentage of mass loss after lyophilization. Microbial load of frozen cells was determined in triplicate using a C6 Accuri flow cytometer (BD Biosciences, New Jersey, USA) as described previously[16].

Cultivation, colony counts and isolation

All post-preservation sample dilution, culturing and isolation steps were performed at 37°C under anaerobic conditions (10% H_2, 10% CO_2, 80% N_2) in a Don Whitley A35 Anaerobic Workstation with HEPA filter (Don Whitley Scientific, Shipley, UK). All dilution and culture media were anaerobically preincubated overnight.

Per post-preservation sample, a dilution series (10^{-1} to 10^{-7}) for each preservation conditions was prepared in PBS and dilutions 10^{-3} to 10^{-7} were plated in triplicate on modified Gifu Anaerobic Medium (mGAM; Hyserve) agar and incubated for 5 days. Plates corresponding to countable dilutions (i.e. plate dilutions producing CFU in the range of 20-200, n=203) were first subjected to CFU counting and subsequently used for isolation. Colonies were selected (i.e. 48 colonies per countable dilution when possible, up to 95 per preservation conditions) for isolation based on morphological differences (i.e. diameter, edge, shape and colour) and were transferred to a 96-well microplate in mGAM broth. Following incubation for 48h, 15% (v/v) glycerol was added and homogenised into each well after which the microwell plates were stored at -80°C to use for identification by partial 16S rRNA gene sequencing. For all dilutions that yielded >100 colonies, one plate was kept aside for harvesting its entire biomass (from here on referred to as cultured fraction) which was resuspended in PBS. Following centrifugation (18,000 xg, 2 min) and removal of

supernatant, these fractions were stored at -80°C for further 16S rRNA gene MiSeq community profiling. The entire experimental design is visualised in Figure 1.

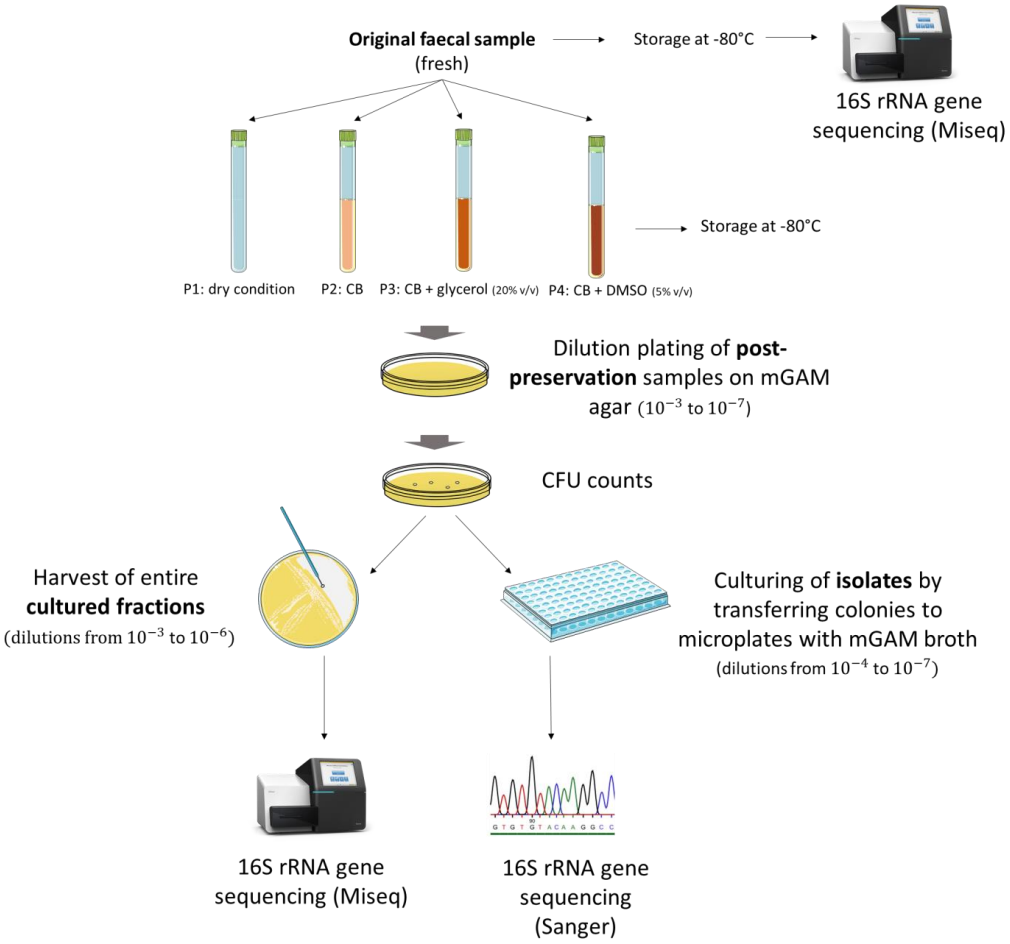


Figure 1. Experimental setup of the study. Aliquots of original fresh samples were used for community profiling and for comparison of post-preservation samples kept at -80°C in four different preservation conditions with or without medium and/or cryoprotectants: dry (P1), CB (P2), CB + glycerol (20%) (P3), CB + DMSO (5%) (P4). Following preservation, sample dilutions for each condition were plated on mGAM and incubated anaerobically. After 48h, CFU count was determined, and colonies were selected from countable plates for isolation in 96-well microplates and subsequent identification by Sanger 16S rRNA gene sequencing. From the same dilution series, plates with high colony numbers were fully harvested as cultured fractions and subjected to MiSeq community profiling.

Community profiling of faecal samples and cultured fractions

Before processing for 16S rRNA gene-based community profiling, cultured fraction pellets were resuspended in 4.5 mL PBS (Sigma Aldrich, USA) and split in 3 aliquots of 1.5 mL. Samples were centrifuged and supernatant was removed prior to storage at -80°C in cryovials. Frozen faecal aliquots (between 150 and 200 mg) and cultured fractions were

subjected to DNA extraction using thePowerMicrobiome DNA/RNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, USA) according to the manufacturer's instructions as described previously[9]. The V4 hypervariable region of the 16S rRNA gene was amplified using forward primer 515F (5' GTGYCAGCMGCCGCGGTAA 3') and the reverse primer 806R (5' GGACTACNVGGGTWTCTAAT 3'), modified with adapters and barcodes[9] . Sequencing was then performed using the Illumina MiSeq platform (MiSeq Reagent Kit v2, 500 cycles, 20% PhiX) according to the manufacturer's specifications to generate paired-end reads of 250 bases in length in each direction. After demultiplexing with sdm as part of the LotuS[132] pipeline without allowing for mismatches, fastq sequences were pre-processed using DADA2 pipeline v1.14.1[133]. The taxonomy was assigned using GTDB release 95[134,135]. For the comparison with FGFP[9] samples, taxonomy classification was also performed using the RDP classifier[136]. For relative microbiome analyses, each sample depth was rarefied to 10,000 reads.

Identification of isolated colonies

From individual bacterial colony suspensions stored in 96-well microplates, 20 µL was transferred into a new microplate and washed with PBS to remove excess medium. Plates were centrifuged for 10 min at 3,000 x g, after which the supernatant was removed. Following a second washing step, pellets were resuspended in 20 µL alkaline lysis solution containing 2.5 mL 10% SDS (Invitrogen, USA), 5.0 mL 1N NaOH (Merck, New Jersey, USA) and 92.5 mL sterile MilliQ per 100mL. Samples were heated at 95°C for 15 min and immediately put on ice for 10 min. Finally, 180 µL of sterile MilliQ water was added and after gentle mixing, samples were centrifuged for 20 min at 3,000 x g and stored at -20°C until further analysis. For identification based on partial 16S rRNA gene sequencing, 2 µL of each extract was used as template for PCR with forward primer 27F (5'AGAGTTTGATCCTGGCTCAG 3') and reverse primer 1492R (5' ACGGCTACCTTGTTACGACTT 3'). Following agarose electrophoresis check, amplicons were sent for 16S rRNA gene Sanger sequencing to Eurofins (Germany). Sequences were taxonomically assigned by GTDB release 95[134,135].

Statistical analyses

All statistical analyses and graphical representations were performed in R using the packages phyloseq[137] vegan[138], CoDaSeq[139] and ggplot2[140]. Observed richness was calculated with

the R package phyloseq. Microbiome variation between individuals was visualised by PCoA using Bray-Curtis distances, only samples with more than 10,000 reads and amplicon sequence variants (ASV) with a relative abundance higher than 0.001 across the dataset were included in the 16S rRNA data analysis. Correlations between continuous variables were analysed using non-parametric Spearman tests.

The contribution of metadata variables to the cultured fraction community variation was determined by distance-based redundancy analysis (dbRDA) on genus-level Aitchison distance (Bray-Curtis distances between samples after rarefication) with the capscale function in the vegan R package[138]. Correction for multiple testing (Benjamin–Hochberg procedure, FDR) was applied and significance was defined at FDR < 0.1. The cumulative contribution of metadata variables was determined by forward model selection on dbRDA with the ordiR2step function in vegan[138], with variables that showed a significant contribution to cultured fraction community variation in the previous step.

Alpha-diversity, number of observed genera, species and ASV (richness) of both the cultured fraction and faecal samples were determined using the vegan R package[138]. Associations between richness and diversity of the cultured fractions and preservation conditions were assessed by fitting GLMs. The model used multivariate analysis assessing the effect of preservation conditions, dilution factors and initial richness (RichnessF) from the faecal samples. The significance of was assessed by performing log-likelihood (χ^2) tests.

$$Nullmodel\ richness = specnumber \sim Dilution + Preservation + RichnessF$$

With specnumber as the number of species found in the sample (richness).

Results

Faecal sample selection based on physicochemical and microbial parameters results in a wide spectrum of covered microbiota compositions

Faecal samples from eleven donors were selected from a larger cohort based on gradients[9] in faecal pH, water activity, moisture content, and microbial load (Supplementary Figure S1.1). The four parameters did not show any significant cross-correlations (Spearman test, p>0.05, Supplementary Figure S1.2). For each of the selected faecal samples, one aliquot was subjected to microbial community profiling in order to obtain a culture-independent baseline of the microbial diversity in each sample. Analysis of these profiles showed that the 11 faecal samples comprised a broad compositional diversity, as evidenced by their microbial community variation when compared to the larger Flemish Gut Flora Project[9] (FGFP) population (n=1,103) (Figure 2A) and by their diverse top 20 genera profiles (Figure 2B). Thus, the sample selection process based on physicochemical and microbiological gradients resulted in a subset of faecal samples displaying a wide range of microbiota compositions.

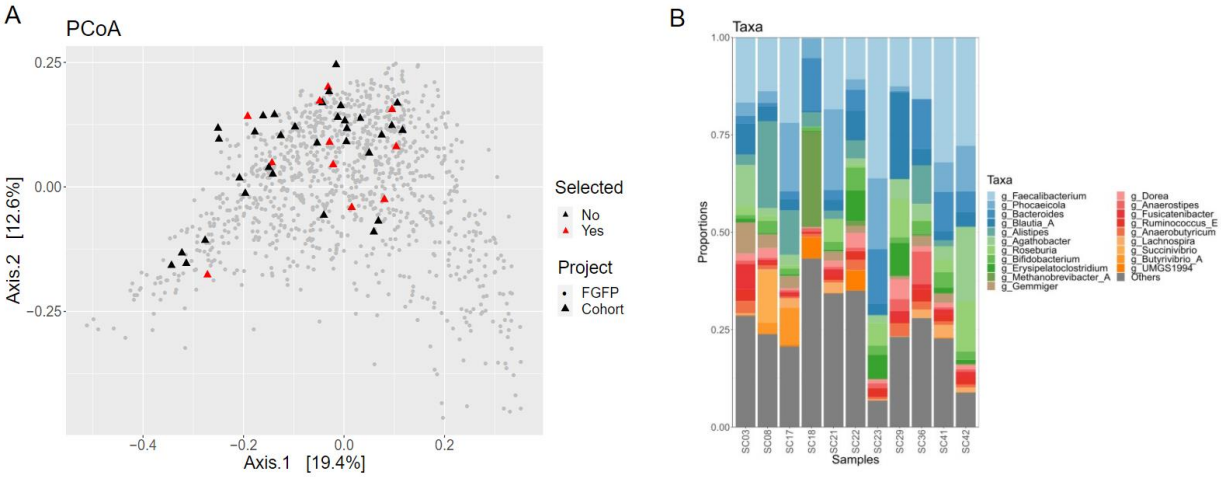


Figure 2. A. Genus-level faecal microbiome community variation, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the original cohort (triangles, n = 51) further subdivided in a subset of 11 samples selected for this study (red triangles) and the non-selected samples (black triangles), as well as samples from the FGFP cohort[9] (dots, n = 1,103). B. Relative abundances of the top 20 genera from the 11 selected faecal samples derived from 16S rRNA gene community profiling.

Quantitative recovery but not overall microbial community composition is impacted by preservation condition in cultured fractions

A first comparison across preservation conditions was based on enumeration of colony forming units (CFU) recovered from dilution plating of post-preservation samples on mGAM. Between conditions, recovery rates ranged from 1.1 x 10^7 CFU/g to 1.9 x 10^{10} CFU/g across all dilutions (countable dilutions ranged from 10^{-4} to 10^{-7}) and from 2.8 x 10^8 CFU/g to 5.2 x 10^9 CFU/g across the most common dilution 10^{-6} (Supplementary Table S1.2, Figure 3A). For dilution 10^{-6}, quantitative recovery from post-preservation samples preserved in the presence of CB medium supplemented with 5% (v/v) DMSO (condition P4) was significantly higher compared to dry preservation (P1) and preservation in CB only (P2), while not significant compared to CB supplemented with 20% (v/v) glycerol (P3) (Kruskal–Wallis with post hoc Dunn test, $p < 0.001$; $p < 0.01$, Figure 3 A).

Next, we explored to what extent preservation condition or any of the other experimental variables affected the overall community composition of the cultured fractions (i.e the harvest of the entire agar plates as illustrated in Figure 1 after culturing post-preservation samples) harvested from the least diluted mGAM plates (n=129). Distance-based redundancy analysis (dbRDA) revealed that sample donor identity, the four physicochemical and microbiological sample selection parameters as well as sample dilution rate all contributed significantly to the differences in overall community composition between the cultured fractions, while sample preservation condition did not (dbRDA, adjusted R^2 range = 2.7–65.5%, FDR < 0.1; Figure 3 C, Supplementary Table S1.3). However, only donor identity and dilution rate had a non-redundant effect on community composition (stepwise dbRDA, total R^2 = 65.8%, Supplementary Table S1.3), with the former driving the highest effect size (Figure 3 B and C).

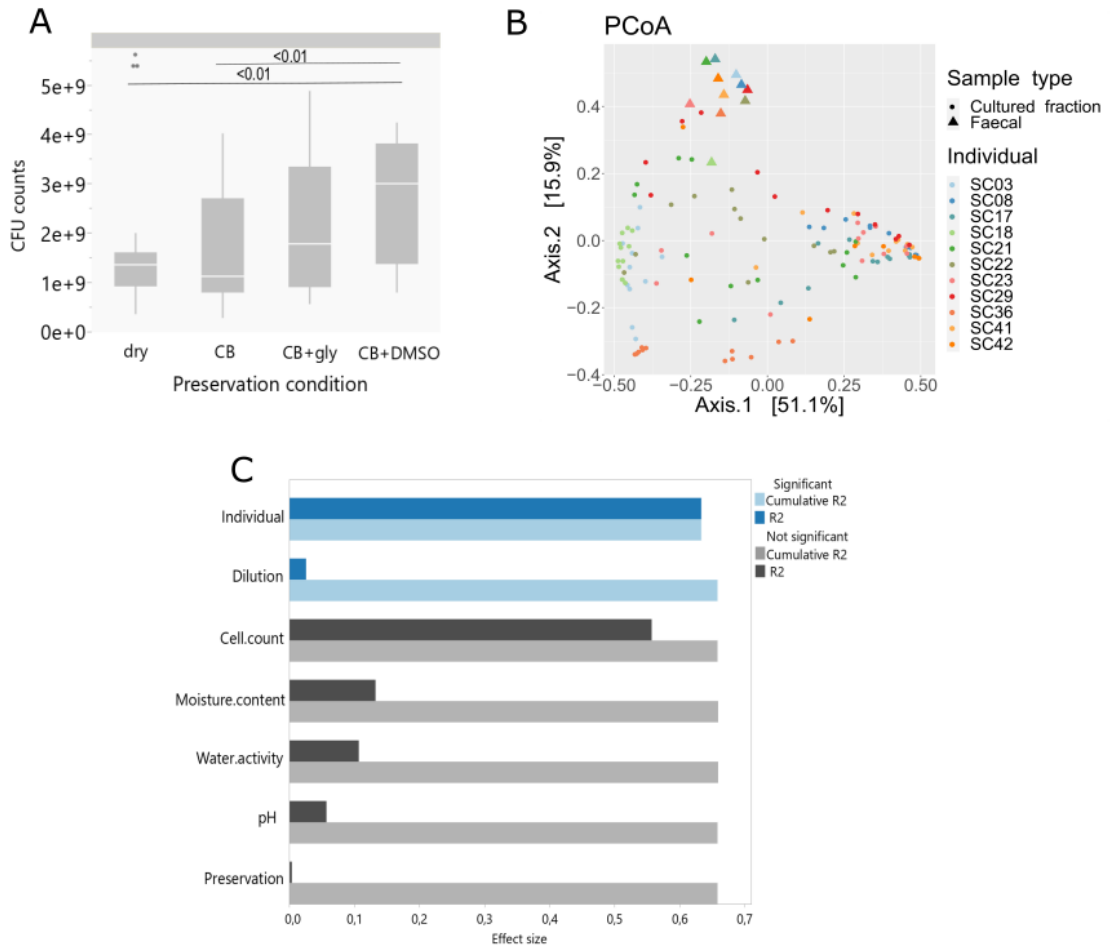


Figure 3: A. CFU counts of cultured fractions obtained from dilution 10^{-6} in triplicate for each preservation condition including all individuals (n= 103), significant differences were observed between condition P4 and conditions P1 and P2, respectively (Kruskal–Wallis with post hoc Dunn test, $p < 0.001$; $p < 0.01$ for both) B. Community variation of cultured fractions and original faecal samples at genus level visualised by principal coordinates analysis (genus level Bray-Curtis distances). Samples were coloured by donor identity and include all preservation conditions and dilutions (n=140, 11 faecal samples and 129 cultured fraction samples). C. Variables contributing most to compositional variation in the cultured fractions (dbRDA, genus level Bray-Curtis distances), either independently (univariate effect sizes in dark blue/grey) or in a multivariate model (cumulative effect sizes in light blue/grey), bars in blue are significant, in grey not significant p<0.001.

Bray-Curtis distances between the original faecal sample and all cultured fractions per individual also did not reveal significant differences between the preservation conditions (Kruskal-Wallis, Dunn's paired test p >0.05) (Supplementary Table S1.4, Figure 3B). Community composition analysis of original samples and their corresponding cultured fractions identified a total of 174 genera at a relative abundance threshold >0.01% of which

the 20 most abundant genera together representing 75.9% of the total abundance (Supplementary Table S1.5). Out of these 20 genera, only 6 were detected in the cultured fractions including the genera *Bifidobacterium, Bacteroides* and *Phocaeicola.* They accounted for 81.4% of the total abundance, compared to 20.7% of the total average abundance in the original faecal samples. For instance, *Bifidobacterium* had an average relative abundance of only 2.9% in the uncultured faecal samples, but co-dominated cultured fractions with an average relative abundance of 39.9%. Likely, the high dominance of a few genera is driving the overall community composition in all cultured fractions and explains the lack of significance between the preservation conditions overall.

Next, we sought to identify if preservation conditions would influence total microbial richness of the recovered cultured fractions. Fitting generalised linear models (GLMs) showed that both sample dilution and richness of the uncultured source samples had a significant effect on the cultured fraction richness at genus level (GLM, $n = 129$, $p<0.005$, Supplementary Table S1.6) but not at species level. Preservation conditions did not have a significant effect on richness at any taxonomic level, independently of the fraction or only the lowest most common dilution 10^{-4} being compared (GLM, $n = 43$, $p>0.1$, Supplementary Table S1.6).

Preservation conditions differentially affect recovery of specific genera

Next, in addition to comparison of overall richness, we also pursued a more narrow approach to explore whether sample preservation conditions affected anaerobic recovery of specific bacterial genera. For this purpose, we compared cultured fractions from the highest most common dilution 10^{-4}, as we previously reported an effect of dilutions. In total, 45 genera were detected at >0.1% abundance in the cultured fractions of sample dilution 10^{-4} in all individuals (Table 1). Presence-absence analysis indicated that twenty-two of the 45 most abundant common genera were recovered in fractions from all preservation conditions, seven from three of the four preservation conditions and seven from two preservation conditions. However, the remaining nine genera were only detected in fractions from one preservation condition: five from dry (P1), two from CB + 20% (v/v) glycerol (P3) and CB + 5% (v/v) DMSO (P4) (Table 1). Overall, the highest number of common genera (i.e. 35/45) were recovered from sample aliquots preserved as CB + 5% (v/v) DMSO (P4) and dry (P1), followed by preservation conditions CB + 20% (v/v)

glycerol (P3) (32/45), and CB only (P2) (30/45) (Table 1). When also taking into account relative abundances, weak significance was only found for the genus *Collinsella* which was recovered more in fractions obtained from condition CB only (P2) compared to CB + 20% (v/v) glycerol (P3), but not compared to other preservation conditions (Dunn's test post-hoc, $p = 0.047$). None of the other genera were significantly associated with a specific condition. Possible explanations for these results could be that most genera were only recovered in one or two preservation condition from a single individual and in low abundance. Nonetheless, the observation that over half of the most common genera were not recovered in all preservation conditions indicates that different preservation methods are needed to recover specific sets of taxa. Thus, while overall richness was not significantly different between preservation conditions, differences in the recovery of individual taxa were frequently observed.



Figure 4. Presence-absence distribution of bacterial genera in cultured fractions (dilution 10^{-4}) of each preservation condition at >0.1% abundance. The coloured bars indicate the number of individuals in which a genus was detected each colour represent one individual.

Parallel use of different preservation conditions can increase taxonomic richness of derived culture collections

Finally, we assessed the effect of preservation conditions on the species richness of bacterial culture collections isolated from cultured fractions. Manual colony picking from plates inoculated with the highest sample dilutions resulted in a collection of 4,088 isolates that were subjected to 16S rRNA gene sequencing. Sequences were obtained for 3,395 isolates, resulting in a median of 82% of isolates sequenced per preservation condition per individual (range between 41 to 100%, Supplementary Table S1.7). Using a read length >500 bp and a sequence similarity >98% as minimal quality requirements, a total of 3,008 sequenced isolates were retained for further analysis.

The 3,008 isolates represented eight different phyla distributed over 54 genera and 104 species, of which the majority belonged to the phyla Actinobacteriota (38.0%) and Bacteroidota (57.1%), and at family level, to the *Bifidobacteriaceae* (27.6%) and *Bacteroidaceae* (41.6%), respectively. Overall, culture collections with highest richness were isolated from samples preserved as dry (P1) (36 genera and 72 species) and CB + 5% (v/v) DMSO (P4) (33 genera and 66 species). Proportional to the number sequenced isolates, the P1 strain set harboured 1.2 to 1.5 times more genera and species than other preservation conditions (Supplementary Figure S1.3 and Supplementary Table S1.8). Twenty-three genera and 43 species were exclusively found in one preservation condition: P1 (n=8; n=16), P2 (n=6; n=12), P3 (n=2; n=3) and P4 (n=7; n=12). Within the limitations of the colony selection process, these results suggest that parallel use of different sample preservation conditions can increase species and genus richness of subsequently generated culture collections.

From the 45 genera collectively recovered in cultured fractions, 23 (51.1%) were not effectively isolated in the colony picking process (Supplementary Table S1.9). For instance, members of the genus *Dialister* were detected in cultured fractions obtained from the lowest dilutions of sample aliquots preserved at any of the four preservation conditions but were never retrieved during the isolation process. Conversely, 30 out of the 55 genera (45.5%) represented in the entire culture collection were not detected in any of the cultured fractions (Supplementary Table S1.9). For example, *Akkermansia* was not detected in any cultured fraction dilution whereas 11 isolates were recovered from 4 individuals in all the tested preservation conditions (P1, n=8; P2-P4, n=1 each). Compared to the 70 genera detected at >0.01% abundance by community profiling of the uncultured faecal samples, 24 genera

(32.3%) were recovered from cultured fractions and a different set of 24 genera (32.3%) were effectively isolated. Conversely, 31 genera represented in the entire culture collection were not detected by metagenomic profiling of the uncultured faecal samples. Possibly, these are low-abundant taxa that were enriched during the cultivation process (Supplementary Table 9). Comparison of taxonomic richness obtained from faecal samples, cultured fractions and culture collections indicates that sample preservation conditions, sample dilution and the semi-random colony selection process may contribute to the observation of missing taxa.

Discussion

Preservation conditions of faecal samples aiming at subsequent isolation and cultivation of gut anaerobes have been poorly studied so far. Here, we investigated the impact of dry cryopreservation versus three cryopreservation medium formulations with or without cryoprotectant on the recovery and isolation of gut microbiota members from 11 human faecal samples. We find that the method of preservation has an important effect on the recovery and isolation of high and low abundant bacterial taxa from faecal samples. Below, we discuss several factors that might have contributed to these differences.

In any comparative study investigating the influence of sample process parameters on the preservation of microbial diversity contained in those samples, careful study design and sample selection is crucial. In this study, selection of faecal samples based on physicochemical and microbiological gradients resulted in a compositionally diverse set of samples that enabled the recovery of many different taxa. The most common preservation method of faecal samples is freezing in dry condition without addition of any medium. In our study, this preservation condition (i.e., P1) as well as the addition of CB medium supplemented with DMSO (i.e. P4) yielded the highest number of recovered common genera in cultured fractions. The addition of CB only (i.e., P2) did not seem to contribute to this effect in cultured fractions nor in isolated subcollections. While CB is a commonly used sample transport medium for maintaining viability of enteropathogens and anaerobic bacteria, it shows lower recovery of enteropathogens when used for cryopreservation storage[141] and is therefore better suited to preserve bacterial viability during short periods

at 4°C[142]. Long-time storage at sub-zero temperatures requires addition of cryoprotectants to minimise cell damage during freezing and thawing. In our study, supplementation of CB with glycerol or DMSO was associated with the exclusive recovery of several genera from cultured fractions (Table 1), while no specific taxa were recovered on CB only.

A number of genera (i.e., *Blautia_A, Clostridium_Q, Enterocloster, Ruminococcus_B,* and *Peptoniphilus_B)* were recovered only under dry preservation conditions, suggesting that CB medium and/or certain cryoprotectants might exert taxon-specific growth inhibition or toxic effects. Compared to glycerol, which has been shown to form ice crystals inside cells potentially leading to cell lysis during thawing, DMSO generally allows better recovery of a wide range of microorganisms [126,143,144]. This was also corroborated in the present study where CFU count-based quantitative recovery from faecal aliquots preserved in the presence of 5% (v/v) DMSO (i.e., P4) was significantly higher compared to P1 and P2 aliquots, and to a lesser extent, P3. Furthermore, two genera (i.e., *Parasutterella and Clostridium_P*) were exclusively recovered in cultured fractions from P4 aliquots. However, it cannot be ruled out that a concentration of 5% DMSO might inhibit recovery of certain other genera, potentially explaining why some taxa recovered exclusively under conditions P1-P3 were not detected in P4 aliquots. If logistically feasible, parallel cryopreservation of sample aliquots under multiple cryopreservation conditions such as dry and CB+DMSO storage, and/or the addition of other cryoprotectants such as trehalose[143,145] might be recommended in order to increase cultured bacterial richness from cryopreserved faecal samples. Moreover, the addition of some compound used in preservation (e.g, DMSO) may be detrimental not only to cells but also by altering gene expression and therefore transcriptomics or other omics data as already described in human cells[146] or fungi[147]. For this reason, more research may be needed to study the impact of such compound for multi-omics studies.

Also when assessing the taxonomic range among bacterial isolates semi-randomly picked from mGAM plates, the combined culture collections derived from dry cryopreservation and aliquots stored in the presence of CB+DMSO yielded the highest number of cultured species, with more taxa specifically isolated from dry cryopreservation. Evidently, the choice of culture medium to compare bacterial recoveries across preservation conditions is

of great importance. To keep the study logistically manageable, we restricted comparisons to only the widely used anaerobic mGAM medium previously shown to enable growth of many gut anaerobes[107,119,131]. Still, 67.9% of the isolates recovered from cultured fractions in our study belonged to just three genera, i.e. *Bifidobacterium* (31.3%)*, Bacteroides* (27.0%) and *Phocaeicola* (9.6%), while in uncultured samples these taxa had a collated relative abundance of only 20.7% (2.9%, 7.0% and 10.8% respectively). Apart from the possibility that part of the cells detected in community sequencing might be dead, damaged or metabolically inactive, it is likely that the nutritional conditions of the mGAM medium favour recovery of a few specific taxa but inhibit or lack an essential growth factor of other highly abundant gut members that remained undetected in cultured fractions. Future study designs including nutritional supplementation of mGAM and/or the use of additional media can be useful to check to what extent the recovery of major missing taxa such as *Faecalibacterium* would have been affected differently by the tested preservation conditions. Next to preservation condition and medium, however, our study shows that also the sample dilution rate co-determines the species richness recovered during isolation. This might be especially relevant for low-abundant species that can be washed out during the dilution process and/or of which the few remaining cells are overgrown by fast-growing species. In addition, it should be kept in mind that other limitations of agar-based isolation approaches such as overgrowth of slow-growing species by faster growing ones and the inability to pick microscopically small colonies might further distort the investigator's view on the culturable diversity of a sample. Single-cell based strategies, ranging from dilution-to-extinction to microfluidic single-cell droplet technologies, suffer much less from these shortcomings and are emerging at a rapid pace[106].

Conclusion

In this study, community profiling of faecal samples and cultured fractions was combined with 16S rRNA gene sequence identification of isolated colonies. Not only did this confirm the complementarity of culturing and metagenomics approaches to cover the largest taxonomic spectrum possible[148], it also enabled us to investigate the potential impact of sample cryopreservation conditions at multiple levels. We demonstrated that parallel use of multiple faecal cryopreservation conditions can clearly benefit the taxonomic depth of gut

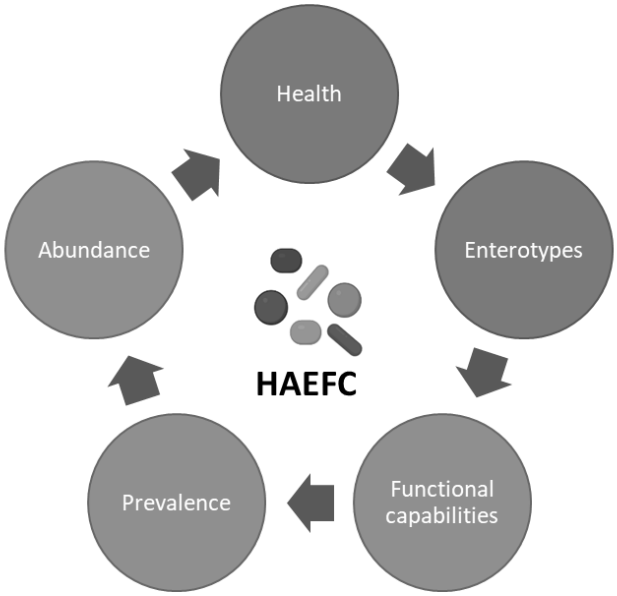
culturomics efforts. Therefore, the current practice of cryopreserving stool samples in dry conditions may need to be complemented with other preservation medium conditions such as CB + DMSO to increase the species diversity of gut-associated culture collections. Moreover, we show that a sample selection strategy based on physicochemical and microbiological gradients is another key contributor in maximizing the recovery of a phylogenetically diverse set of bacterial taxa from human stool samples.

Chapter III

Defining a health-associated functional core of the human gut microbiota

Parts of this chapter are being prepared for publication:

Anaïs Biclot; Geert R.B. Huys; […] Jeroen Raes. Design and assembly of defined human gut microbiota core communities.


Health
Enterotypes
Functional capabilities
Prevalence
Abundance
HAEFC

Abstract

Simplifying the complex ecosystem of the human gut microbiota is crucial to gain a deeper understanding of interactions within gut communities. Previous studies have focused on selecting highly prevalent and abundant taxa in a population to identify the core members of the gut microbiota or on selecting members based on a desired function. However, none have looked at designing communities based on gut community types in addition to health-associated species part of a taxonomic and functional core.

This study demonstrated a novel approach in designing a health-associated enterotype functional core. The selection of preliminary core taxa was based on gut microbiome profiling data from a cohort of 1,106 individuals and considered both the abundance and prevalence of members within the healthy part of the cohort in addition to the enterotypes. Then, we defined their metabolic capabilities in silico using publicly available data to define cores that collectively encompass the major trophic levels in the gut ecosystem. Full metabolic characterisation was performed for each selected species using both in silico and in vitro data from literature, and functional clusters were found within the cores.

The study found a high level of redundancy within the cores, which could lead to increased competition, particularly in one cluster. However, potential cooperation was also observed, with several highly cooperative species in the health-associated enterotype functional cores, including *Dorea longicatena, Collinsella. aerofaciens,* and *Ruminococcus bromii*. This study provides a first step in designing health-associated enterotype functional cores and provides a deeper understanding of the core species ecological niche, which can be used for the in vitro assembly of the core communities and for the evaluation of potential competition and cooperation at the community level.

Introduction

Gaining a deeper understanding of the functions and interactions of gut microbial communities requires simplifying this complex ecosystem into defined, simple consortia. Current approaches to select core species for building such consortia involve selecting highly prevalent and abundant taxa from a complex community. The core microbiome has been defined as representing a selection of taxa in common among the microbiomes of all humans[58]. Core members of the gut microbiota have mostly been studied in Western populations, and all studies share a similar core microbiome at genus level[9,10,58].

An alternative approach to select a defined community is to select members based on a desired function, such as the production of a specific metabolite e.g., butyrate[149] or propionate[150]. In this approach, the optimal community is not necessarily composed of common taxa, but rather those that will collectively yield the desired product or function. In an attempt to combine both approaches, a recent study screened for both prevalence of species in the population as well major metabolic routes these species could use for conversion of dietary carbohydrates to SCFAs (here propionate and butyrate) by using in silico tools and publicly available genomes[151].

The aim of this study is to design a **health-associated functional core** (HAFC) with members from the human gut microbiota and define their metabolic capabilities in silico using publicly available data on these species to assemble an in vitro synthetic community of core taxa that collectively encompass the major trophic levels in the gut ecosystem. In addition to taxonomic cores and targeted functional profiles, HAFC designs will also take into account structural variation among human gut communities as revealed by the definition ofenterotypes[14]. Moreover, stratification of human gut microbiomes in enterotypes has been linked to health and disease. One specific enterotype, *Bacteroides* 2 (Bact2), has been associated with gut dysbiosis and an increased incidence of multiple disease conditions[15,18]. Therefore, a cohort of over 1,000 individuals[9] was subdivided into a healthy group as well as the different community types to investigate if each had specific core members. As enterotypes have been found to exert different functions, notably in their saccharolytic, proteolytic and lipolytic profiles[152], the inclusion of enterotyping data adds a novel step that further finetunes HAFC designs towards subject-specific gut ecosystems. This way, comparison of these cores to the healthy core resulted in the design of **health-**

associated enterotype cores (HAECs)**.** Finally, the metabolic capabilities of the core species were investigated to ensure that the main metabolic functions of the gut microbiota were present to build **health-associated enterotype functional cores** (HAEFC).

Methods

Health-associated core selection

In order to select core taxa, we used 16S rRNA gene community profiles of a large cohort of human volunteers containing more than 1,000 individuals from the Flemish gut flora project[9] (FGFP (n=1,106)). Using this cohort, core taxa were first defined with a 90% prevalence threshold in the entire population at genus level with a detection threshold of 0.001%. Likewise, also the 15 highest average abundant taxa (top 15) were retrieved from the dataset, and these taxa were compared to the ones retrieved using the prevalence threshold. The cohort was split into eight groups: i) the full cohort (n=1106); ii) the healthy subset (defined below) (n=203); iii) the non-healthy subset (entire cohort but healthy) (n=903); each of the enterotypes: iv) *Bacteroides* 1 (Bact1, n=418), v) *Bacteroides* 2 (Bact2, n=155), vi) *Prevotella* (Prev, n=172), vii) *Ruminoccocus* (Rum, n=358); and viii) the full cohort excluding Bact2 individuals (n=948). The "healthy" subset only contained individuals without the following medical conditions: celiac disease, inflammatory bowel disease (IBD) (including both Crohn's disease and ulcerative colitis), irritable bowel syndrome, colorectal cancer (CRC), and diabetes (Type 1 and 2)), no loose stools or constipation ($1 < \text{Bristol Stool Score (BSS)} < 6$), a BMI below 25, without inflammation (C-reactive protein; $\text{CRP.mg.L}^{-1} < 50$), age between 25 and 45, no antibiotics used in the last 3 months and not belonging to Bact2 enterotype (as it is linked to inflammation[15]). Finally, the eight taxonomic cores obtained were defined at genus level and compared to work previously published[9,10,153–155]. From the eight cores, the three enterotypes cores (all but Bact2) and the healthy core were further investigated. These four cores were further defined at species level with a prevalence threshold of 60% within the entire population and a detection threshold of 0.001%. At this step, the Amplicon Sequence Variants (ASVs) not assigned to a species with Ribosomal Database Project (RDP Release 11, Update 5[136]) were aligned to the EZ Biocloud database[156]. The prevalent species were then matched with the previously selected core genera to define, to have four species-level cores. The enterotype cores were

compared to the healthy core to remove any genus not associated to health. The three enterotype-specific cores were therefore defined as **health-associated enterotype cores** (HAEC).

Additionally, the metabolic potential of the species contained in the HAECs was investigated. Both their ability to produce short-chain fatty acids (SCFAs) and to degrade macro-molecules such as mucin were investigated. This enabled an overview of the functional potential of the species contained in the three HAEC to encompass the major trophic levels in the gut ecosystem. These three cores were therefore defined as **health-associated enterotype functional cores** (HAEFCs).

Metabolic capabilities of the core

The metabolic capabilities of the selected core species were investigated at different levels. First, an extensive literature review was conducted to find any in vitro data of tested metabolic capabilities in different experiments. Secondly, their potential metabolic capabilities were analysed using in silico tools based on type strains or -if not available- other strain's genomes from the Integrated Microbial Genome (IMG) database[157,158]. More details on the genomes of which functions were retrieved from IMG are listed in Supplementary Table S3.1.

Table 1: List of the strains genomes retrieved from IMG

Phylum	Family	Species	Strain ID	taxon_oid
Actinobacteria	*Coriobacteriaceae*	*Collinsella aerofaciens*	ATCC 25986	640612206
Bacteroidetes	*Bacteroidaceae*	*Bacteroides uniformis*	DSM 6597	2693429911
Bacteroidetes	*Bacteroidaceae*	*Phocaeicola dorei*	DSM 17855	642979370
Bacteroidetes	*Bacteroidaceae*	*Phocaeicola vulgatus*	ATCC 8482	640753008
Bacteroidetes	*Barnesiellaceae*	*Barnesiella intestinihominis*	YIT 11860	2529292932
Bacteroidetes	*Odoribacteraceae*	*Odoribacter splanchnicus*	1651/6	649633078
Bacteroidetes	*Prevotellaceae*	*Prevotella copri*	DSM 18205	2562617166
Bacteroidetes	*Prevotellaceae*	*Prevotella lascolaii*	KHD1	2871890851
Bacteroidetes	*Rikenellaceae*	*Alistipes onderdonkii*	WAL 8169	2517572213
Bacteroidetes	*Rikenellaceae*	*Alistipes shahii*	WAL 8301	650377904
Bacteroidetes	*Rikenellaceae*	*Alistipes timonensis*	DSM 25383	2693429854
Bacteroidetes	*Tannerellaceae*	*Parabacteroides merdae*	NCTC 13052	2811995281
Firmicutes	*Lachnospiraceae*	*Agathobacter rectalis*	ATCC 33656	644736367
Firmicutes	*Lachnospiraceae*	*Anaerostipes hadrus*	SSC/2	650377990
Firmicutes	*Lachnospiraceae*	*Blautia obeum*	ATCC 29174	640963024
Firmicutes	*Lachnospiraceae*	*Blautia wexlerae*	DSM 19850	2528768167
Firmicutes	*Lachnospiraceae*	*Coprococcus catus*	GD/7	650377925
Firmicutes	*Lachnospiraceae*	*Coprococcus comes*	ATCC 27758	643886116
Firmicutes	*Lachnospiraceae*	*Dorea formicigenerans*	ATCC 27755	641736133
Firmicutes	*Lachnospiraceae*	*Dorea longicatena*	DSM 13814	640963046
Firmicutes	*Lachnospiraceae*	*Fusicatenibacter saccharivorans*	MSK.18.88	2920298691
Firmicutes	*Lachnospiraceae*	*Roseburia faecis*	M72	2706794656
Firmicutes	*Oscillospiraceae*	*Agathobaculum desmolans*	ATCC 43058	2561511082
Firmicutes	*Oscillospiraceae*	*Faecalibacterium duncaniae*	A2-165	645951831
Firmicutes	*Oscillospiraceae*	*Gemmiger formicilis*	ATCC 27749	2590828806
Firmicutes	*Oscillospiraceae*	*Intestinimonas butyriciproducens*	DSM 26588	2756170247
Firmicutes	*Oscillospiraceae*	*Ruminococcus bromii*	ATCC 27255	2857962555

Functional clusters

To determine functional clusters, the KEGG orthology (KO) annotations of the genomes described in Table 1 were used. Using Spearman correlation, the genomes were correlated based on their KO annotations. A Spearman correlation $\rho > 0.62$ was kept to build the clusters. Then, still using the KO annotations of the strains, all the genomes were run through the genome metabolic modules (GMMs) as previously described[152]. These modules describe enzymatic pathways annotated using KEGG Orthology (KO) as described previously. The coverage of each module encoded in a GMM was calculated as the number of steps for which the KO was found in a genome, divided by the total number of steps constituting the pathway. As a single enzymatic activity can be encoded by several orthologues, GMMs include variants of a similar metabolic conversion with, in most cases,

identical input/output compounds. GMMs presence/absence was identified with a detection threshold of more than 66% coverage to provide tolerance to miss-annotations and missing data in incomplete (draft) genomes. The KO from all genomes was used to create a correlation matrix between the different species using Spearman correlation (cor function from the stats R package). The correlation with a minimum of 0.62 were then visualised using the R package qgraph.

In silico competition and cooperation

To ensure that the species would cooperate more than compete based on their metabolic niche, a reverse ecology analysis[159] was performed using genomes available on IMG with their KO annotations. A reverse ecology approach is using the genomic features of species to infer the ecology of a community, for example interactions. As described previously[159], this approach uses genome-scale metabolic network models that in this study were built from genomes retrieved from IMG (Table 1). Then, functional annotated genomic data is used to reconstruct the metabolic network for each species. A set of seed metabolites is then used to identify which metabolites are needed by each species from its surrounding. Based on each species seed set, two interaction indices, competition and complementarity index are calculated for each pair of species, where the competition index is calculated when two species can use the same seed metabolite and the cooperation index when a species can use a seed metabolite produced by the other. The results of the analysis were filtered to two cut-offs, 0.75 for the competition index and 0.25 for the cooperation index and visualised using the R package qgraph.

Species metabolic ID

The in vitro metabolic ID was based on the literature searched using the pubmed.gov database with the species name as search words in the title: query as "*Anaerostipes hadrus* [Title]". From this search, papers mentioning metabolism using isolated strains were read to retrieve information about their metabolism studied in vitro. If the search was too broad, an additional search was performed with query "species AND substrate [Title]" (i.e, "*Anaerostipes hadrus* AND mucin [Title]"). Using the information from the modules as well as the literature review, the metabolic ID was constructed with a presence/absence system. "0" shows that no module was assigned in the reference genome of the type strain or that

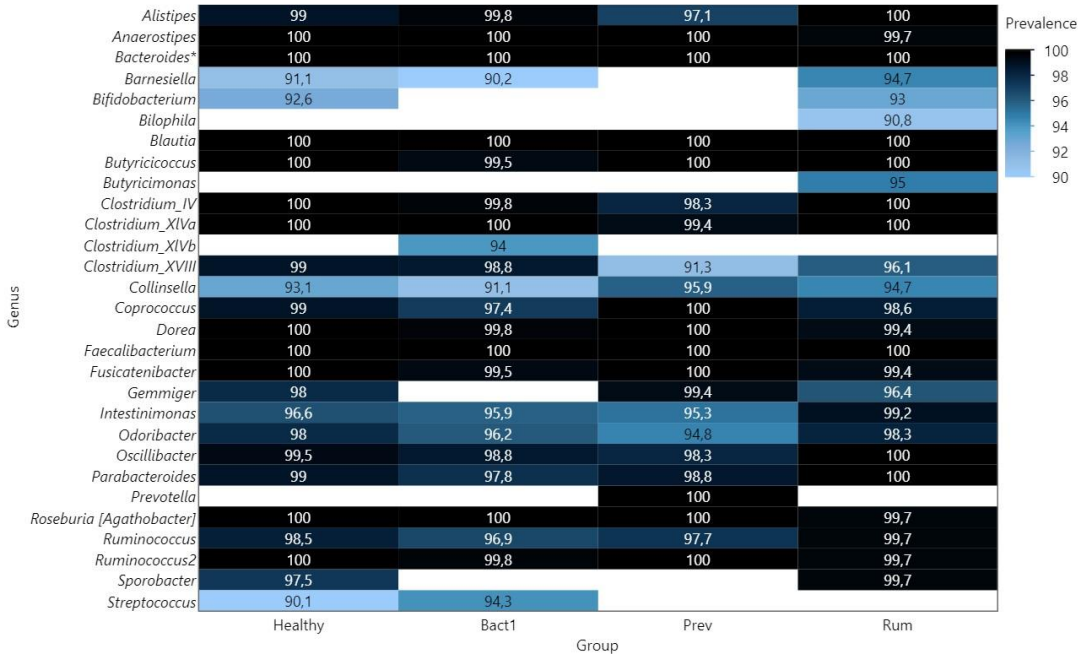
the substrate was tested in vitro but was negative. "1" shows the presence of the module in the reference genome, or the mention of the substrate use, or product produced in the literature. NA means no positive nor negative information was retrieved from the literature review. The second part of the metabolic ID was based on the in silico analysis done on the GMMs.

Results

Building a health-associated core for Prevotella and Bacteroides 1 enterotypes

First, the selection of a core was performed based on the Flemish Gut Flora Project cohort (FGFP; n=1106)[9] using a prevalence threshold of 90% as well as the top 15 highest average abundance taxa, both at genus level. First, the cohort was divided into eight groups: (i-iv) the four enterotypes *Bacteroides* 1 (Bact1), *Bacteroides* 2 (Bact2), *Prevotella* (Prev) and *Ruminococcus* (Rum), v) the full cohort with and vi) without the Bact2 enterotype (as it is linked to inflammation[15]) vii) healthy subset (as described in the methods); and viii) a non-healthy subset (defined as all but healthy people). While the human gut microbiota core is usually defined in the literature at genus level, in order to assemble a synthetic community of core taxa it is necessary to know which species are contained in this core beyond genus level. Next, only the enterotype cores (all but Bact2) and the healthy core were further investigated to select core species. Core species could be selected for most genera with a threshold prevalence at 60% although the 16S rRNA gene sequencing yielded short reads (~250bp). Only genera with known species were selected for the final core list, as some ASVs were assigned to uncultured or undescribed species (e.g Clostridium_XlVa, Figure 1 A and B).

A.



B.

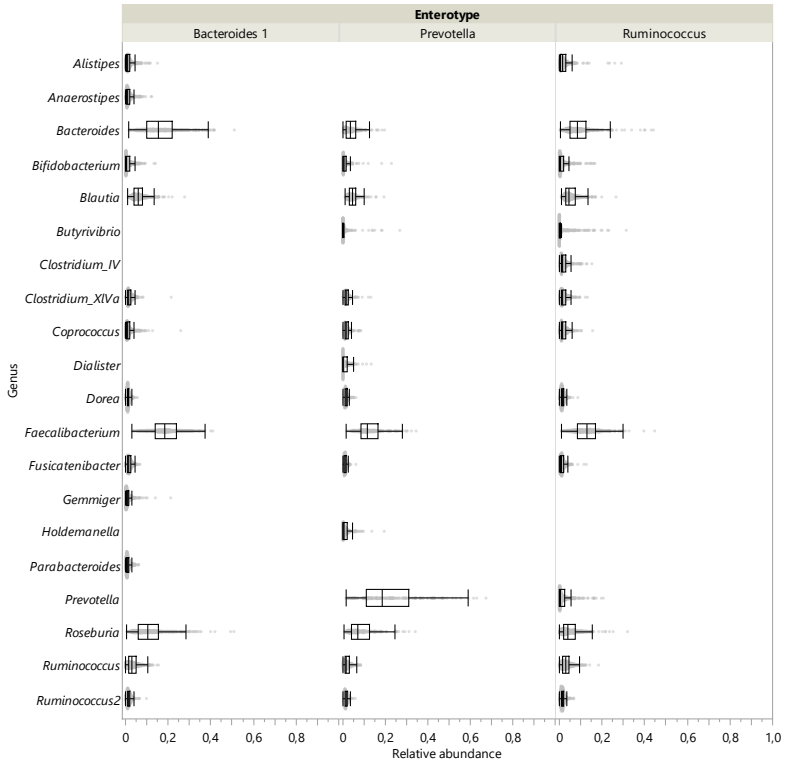


Figure 1. A. Prevalence of core genera (>90% prevalence) for all enterotypes and healthy group at genus level. Healthy = healthy subset (n=203), *Bacteroides* 1 (n=418), *Prevotella* (n=172) and *Ruminococcus* (n=358). B. Differences in abundances at genus level of the top 15 taxa in each of the three enterotypes: *Bacteroides* 1 (n=418), *Prevotella* (n=172) and *Ruminococcus* (n=358).

The **enterotype cores** were overall consistent with previous findings in literature[9,10,153–155] as all genera found in at least 4 of the 5 studies were also found in our analysis (Supplementary Table S3.2). However, to date, no studies have attempted to define core communities in different enterotypes. Our results revealed clear differences between the enterotype cores, both in terms of prevalence and abundance, meaning that a given set of prevalent taxa will not be present at the same abundance in all enterotypes (Figure 1B). The abundance is defined as a subset of the 15 taxa with highest average abundance overall (top15). Some taxa, such as *Barnesiella,* were not prevalent in all enterotypes (i.e., absent in the Prev enterotype (prevalence <90%)), while others, such as *Alistipes*, were highly prevalent (>99%) in most enterotypes, except in Prev with a lower prevalence at 97%, and not in the top15 genera. In terms of abundance, *Blautia* was present in the top15 most abundant with 100% prevalence in all four enterotypes but was present in significantly different abundances in different enterotypes (Figure 1 B) with higher abundances in Bact 1 and Bact2 and lower in Prev and Rum (Krukal-Wallis test post hoc Dunn test $p < 0.001$, Supplementary Table S3.3).

Moreover, only a few studies have investigated or proposed core communities at species level [153–155], mainly due to the limited taxonomic resolution offered by short 16S rRNA gene sequencing amplicons[160]. Nonetheless, for most genera, it was possible to identify the most prevalent species in our dataset and to filter out unnamed species. At species level, more differences were observed compared to genus level, such as in the *Bacteroides* genus where *B. uniformis* was highly prevalent in all enterotypes (although in different abundances) while *B. dorei* was only prevalent in the Bact1 enterotype. Among the three enterotypes, Bact1 and Prev represented two distinct cores comprised of different species and significant differences in abundance compared to the Rum enterotype. Thus, only genera present in either Bact1 or Prev were kept for further analysis and compared to the healthy core (Figure 1A) to ensure that the selected Bact1-like and Prev-like cores were **health-associated enterotype cores** (HAECs) (Table 2).

Table 2. Final core species selection for Bact1 and Prev enterotypes based on in silico analysis and literature review. In green, species present and in black absent in either of the HAEC.

Phylum	Species	Community Bact1	Community Prev
Actinobacteria	*Collinsella aerofaciens*	●	●
Bacteroidetes	*Alistipes onderdonkii*	●	●
Bacteroidetes	*Alistipes shahii*	●	●
Bacteroidetes	*Alistipes timonensis*	●	●
Bacteroidetes	*Bacteroides/Phocaeicola dorei*	●	●
Bacteroidetes	*Bacteroides uniformis*	●	●
Bacteroidetes	*Bacteroides/Phocaeicola vulgatus*	●	●
Bacteroidetes	*Barnesiella intestinihominis*	●	●
Bacteroidetes	*Odoribacter splanchnicus*	●	●
Bacteroidetes	*Parabacteroides merdae*	●	●
Bacteroidetes	*Prevotella copri*	●	●
Firmicutes	*Anaerostipes hadrus*	●	●
Firmicutes	*Blautia obeum*	●	●
Firmicutes	*Blautia wexlerae*	●	●
Firmicutes	*Agathobaculum desmolans*	●	●
Firmicutes	*Coprococcus catus*	●	●
Firmicutes	*Coprococcus comes*	●	●
Firmicutes	*Dorea formicigenerans*	●	●
Firmicutes	*Dorea longicatena*	●	●
Firmicutes	*Agathobacter rectalis*	●	●
Firmicutes	*Faecalibacterium duncaniae*	●	●
Firmicutes	*Fusicatenibacter saccharivorans*	●	●
Firmicutes	*Gemmiger formicilis*	●	●
Firmicutes	*Intestinimonas butyriciproducens*	●	●
Firmicutes	*Roseburia faecis*	●	●
Firmicutes	*Ruminococcus bromii*	●	●

From composition to metabolism: three functional clusters within the HAECs species

Next, we assessed the metabolic potential in silico of the selected core species (Table 2), based on publicly available data. The metabolic capabilities of Bact1- and Prev-specific cores were extracted from the available genome information and their KO annotations. This in silico method provides a broad view of the cores' potential in terms of substrate utilisation and metabolic output. By using the KO annotations of the publicly available genomes, along with the GMMs, functional clustering of species was established. Three clusters (1-3) with a strong Spearman correlation ($\varrho > 0.62$, Supplementary Figure 1) were identified. **Cluster 1** is dominated by the order Bacteroidales and has 17% of all present GMMs in common. A wide range of substrate degradation pathways are present (e.g., starch, mucin, pectin…) as well as lactate, succinate, acetate and propionate production. In

Cluster 2, which includes species of the genera *Coprococcus, Blautia* and *Dorea*, 23% of the GMMs are common to all species. GMMs shared by more than 80% of the cluster are related to the degradation of several substrates (such as fructan, starch and some disaccharides) as well as the utilisation and production of several metabolites such as acetate, lactate and ethanol. In **Cluster 3,** comprising the genera *Faecalibacterium* and *Gemmiger*, 76% of the GMMs are shared by all members. The cluster contains a few specific substrate degradation pathways such as pectin and cellobiose and groups species that produce butyrate with lactate consumption (Figure 2).

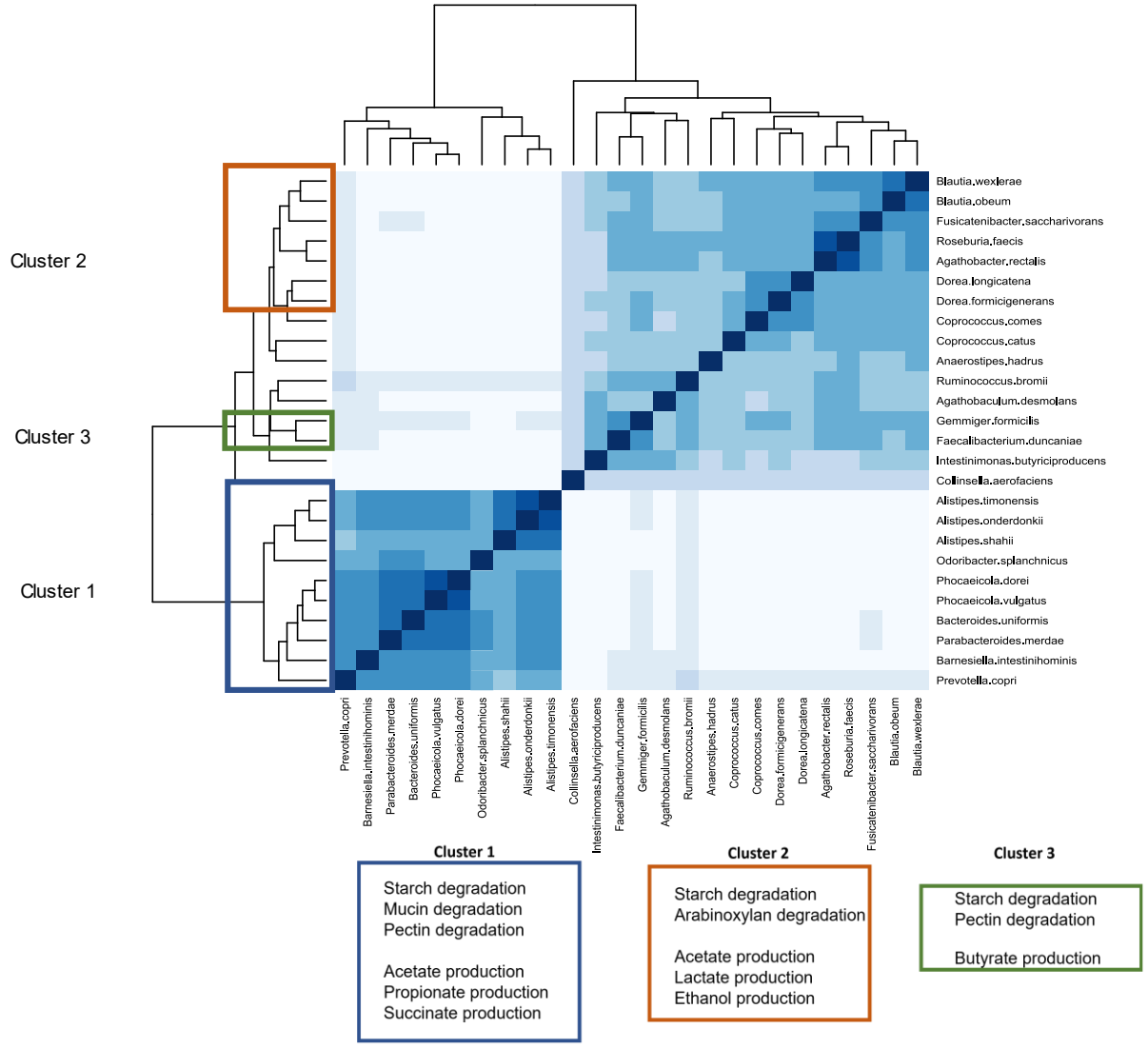


Figure 2. Functional correlation of genomes with KOs (Spearman correlation). The modules in common in at least 80% of the genomes are found at the bottom of the heatmap per cluster (clusters based on Spearman's $\varrho > 0.62$).

Remaining taxa outside of these clusters such as *Intestinimonas, Agathobaculum, Collinsella, Anaerostipes, Ruminococcus* and *Coprococcus catus* shared several modules found in the clusters as well as additional modules. For instance, *Cop. catus* exhibited different modules from *Cop. comes* including butyrate production, whereas *Cop. comes* can degrade starch and fructan This information provides important knowledge on the functional potential of the HAECs which will impact community behaviour and assembly in vitro.

The metabolic potential of the HAECs species

Next, an in-depth literature review was performed to retrieve information on the metabolic capabilities of each species in vitro. As in silico predictions do not always reflect in vitro situations, this analysis allowed a more comprehensive overview of the species metabolic potential. By combining the information of both in vitro and in silico analyses, a metabolic ID for each strain was generated, which includes substrate utilisation, intermediate product usage and product release (Figure 3).

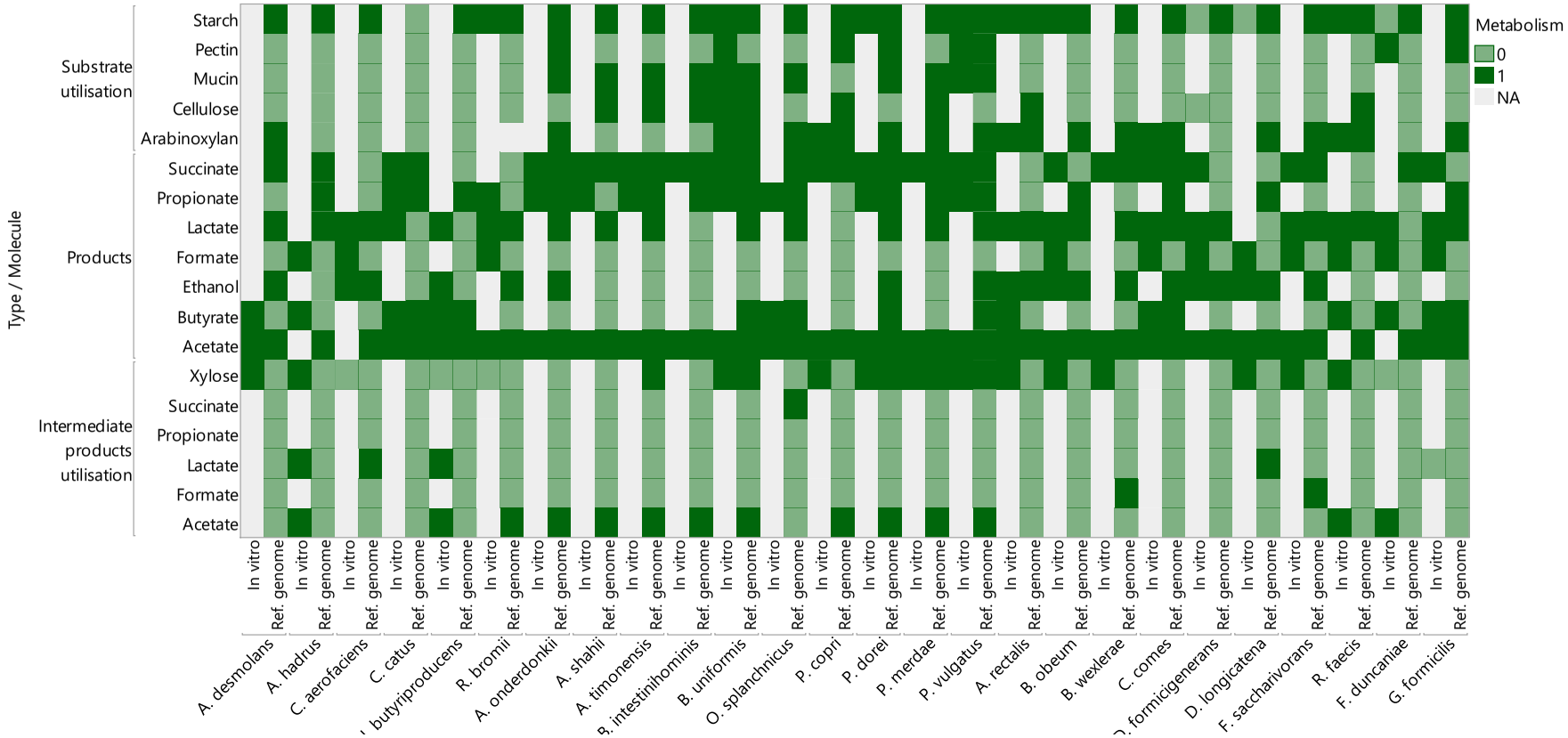


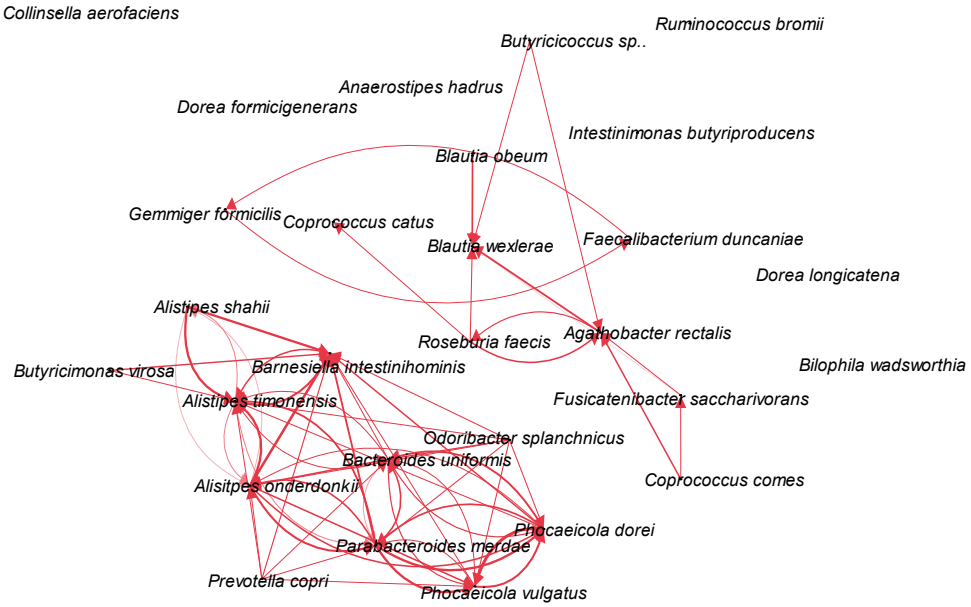
Figure 3. Metabolic ID of each strain based on i) in vitro literature (in vitro) ii) predicted metabolism from reference genomes (Ref. genome) using the GMMs. '0' (green stripes) shows that no module was assigned in the reference genome of the type strain or that the substrate was tested in vitro but was negative. '1' (green) shows presence of the module in the refence genome, or the mention of the substrate use, or product produced in the literature. NA (grey) means no positive nor negative information was retrieved from the literature review. The cluster each species belongs to is found at the bottom (1 to 3), 0 means not in a cluster.

The metabolic functions reported in the in vitro literature review were distinct from those found in silico. This disparity can be attributed to the fact that in silico predictions provide the entire potential of the genome, while in vitro metabolism is heavily dependent on the culture conditions (e.g., medium or pH). As a result, some potential functions found in the genome may only be utilised by the bacteria under specific conditions, such as the production of lactate or propionate based on the substrate availability. On the other hand, in silico analysis depends on the assembly and annotation quality of genomes, which may result in the omission of some functions. For instance, the ability of *Collinsella aerofaciens* to produce formate in vitro was not predicted by in silico analysis. Finally, strain specificity should always be kept in mind in this type of comparisons. While the metabolic identification based on the genomes was performed using type strains, it is possible that in vitro data were obtained from other strains, further contributing to potential lack of congruence between both approaches.

The HAECs species have high competition and cooperation potential

Finally, potential competition and cooperation between species were assessed in silico using reverse ecology analysis[159] (Supplementary Table S3.3). This tool uses genome-scale metabolic network models constructed from genomes. Functional annotated genomic data was used to reconstruct the metabolic networks for each species. Seed metabolites were used to determine the metabolic requirements of each species and calculate interaction indices, including competition and complementarity indices, for species pairs based on their seed sets. Using this technique, we calculated a competition index based on mutual use of the same metabolites, and a cooperation index based on shared metabolites. The analysis was performed using genomes from the IMG database, which provided access to the KO information. Cluster 1 showed 57% of competition indices (51/90) >0.75 between its members (Figure 5 A). This cluster seems to show a very high functional redundancy, which could lead to increased competition between taxa. In comparison, Cluster 2 exhibited about 18% (10/56) competition indices >0.75 (Supplementary Figure S3.2). Noteworthy, more competition was observed between closely related taxa (e.g., within *Bacteroides* and *Blautia*, and between *Roseburia faecis* and *Agathobacter rectalis).* On the other hand, *D. longicatena* was identified as a hub within the core with 14 cooperation partners out of the 25 species (56%) (Figure 5 B).

A.



B.

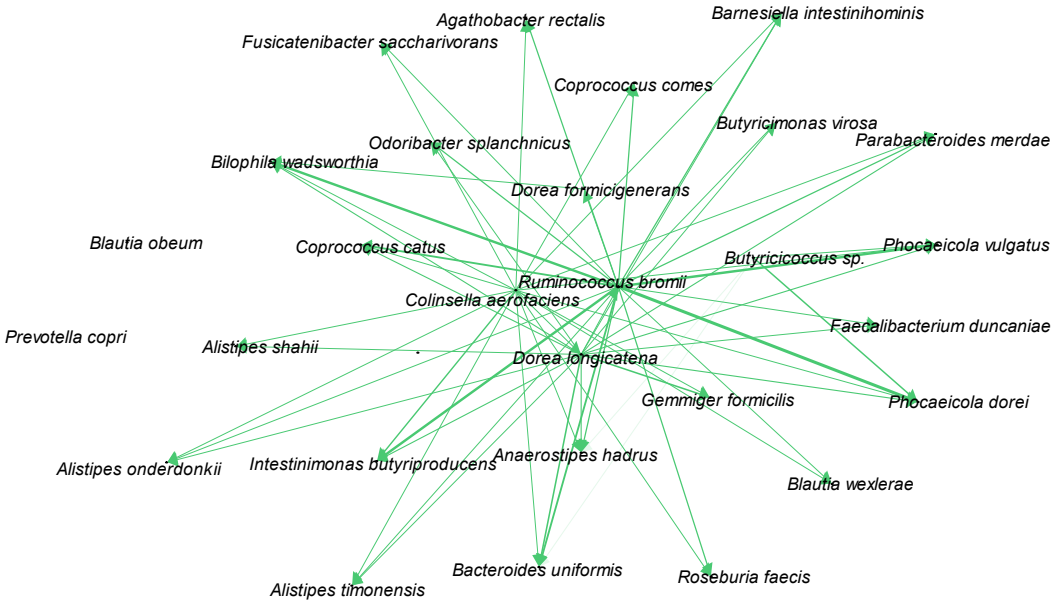


Figure 5 Reverse ecology analysis for all the taxa selected in the cores with A. the competition index > 0.75 shown and B. the cooperation index > 0.25 shown.

Discussion

The study of species interactions in the human gut microbiota using defined communities is becoming increasingly important for the development of new biotherapeutics. In this process, it is crucial to choose species that represent common gut members and functions in order to assemble a community that is ecologically meaningful. The two **health-associated enterotype functional cores** (HAEFCs) selected in this study consisted of bacterial taxa prevalent and/or abundant bacteria in the gut of a healthy western cohort of 1,106 individuals. This study went beyond previous studies by examining the core not just in terms of prevalence and abundance[9,10], but also by focusing on the functional core of different enterotypes within the healthy population, ensuring the presence of essential functions from the gut ecosystem. While the core species were defined for Bact1, Prev and Rum enterotypes, the functional analysis was only performed for Bact1 and Prev. Defining the core microbiota has been the subject of many studies starting from the Human Microbiome Project[58], but studies overall find different core microbiomes even though some taxa overlap, mostly at genus level[161].

These differences led to several hypotheses about the core microbiome, i.e., i) they might only be present in specific human sub-populations possibly associated with certain geographical regions, dietary types, lifestyles etc., ii) that cores may be more easily distinguishable at higher taxonomic ranks but not at species level, given that individuals commonly share the same genera but harbour different species, or iii) that the core microbiota is influenced by functional redundancy[161]. These hypotheses offer diverse perspectives on the delineation and ecological significance of the core microbiome in humans, some of which could be complementary. Most of the previously reported cores rely on prevalence and/or abundance of taxa from 16S rRNA gene sequencing datasets that are usually derived from Western cohorts[9,10,153,162]. As this has generated cores with multiple compositions, in this study we therefore focused on examining the presence of cores within enterotypes as proxies for ecologically and metabolically different community types. From our analysis, we found that most genera were in common between different enterotypes (Bact1, Prev and Rum), including genera also frequently reported in other studies such as *Bacteroides* or *Faecalibacterium.* On the other hand, our analysis also showed that some genera were associated with specific enterotype cores, e.g., *Bilophila* in Rum or *Prevotella* in Prev. Additionally, on top of specific prevalence patterns, a few of the common genera such as *Alistipes* displayed differences in abundances were observed between the different

enterotypes. However, identifying core taxa at the species level posed a greater challenge. To address this issue, we lowered the prevalence threshold from 95% to 60% for the selection of species to be analysed in vitro. Overall, our core taxa differed from previously defined core communities, as they targeted more distinct subsets of the population i.e., enterotypes.

In a next step, selected core species were subjected to metabolic characterization using both in silico data and in vitro data from literature. This analysis was performed only on the Prev and Bact1 enterotypes as the two driving genera, *Bacteroides* and *Prevotella* respectively, are hypothesised to be antagonistic[163]. The functional information obtained was then used to delineate functional clusters within the cores and to assess the core functions performed by the constituting species in each of these clusters. The results revealed a high level of functional redundancy, particularly in cluster 1, where more than half of the members exhibited a competition index >75% indicating increased competition within the community in vitro. Conversely, lower levels of competition were observed in clusters 2 and 3. The functional information of the cores we acquired in this way was not only important for the experimental design of in vitro studies, but it also provided broader insights on the concept of functional cores as alternative approach to taxonomic cores as previously discussed[10,151,161]. As different individuals may harbour different sets of species, defining functional cores may be a useful future approach, keeping in mind that enterotype-specific functional cores should be taken into account given their overall distinct functional profiles[152].

Conclusion

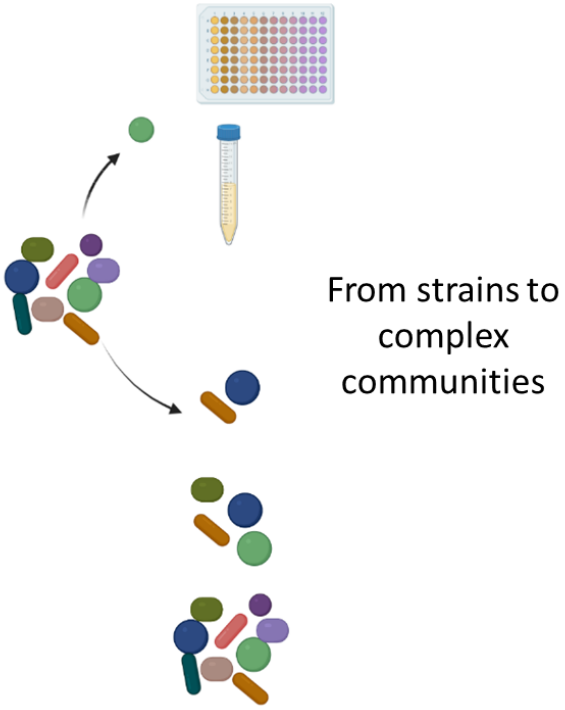
This study reports on a novel approach in designing a **health-associated enterotype functional cores**. While the selection process was largely based on in silico analysis, it provided a deeper understanding of the predicted positive and negative interactions between HAEFC member taxa. This type of information is crucial for the in vitro assembly of synthetic HAEFC communities, e.g., in relation to finding a common growth medium.

Chapter IV

Assembly and perturbation of defined minimal microbiota communities

Parts of this chapter are being prepared for publication:

Anaïs Biclot; Geert R.B. Huys; […] Jeroen Raes. Design and assembly of defined human gut microbiota core communities.


From strains to
complex
communities

Abstract

Dysbiosis in the gut microbiota has been associated with a range of diseases, including gastrointestinal disorders and neurodegenerative conditions. Understanding the dynamics and interactions within the gut community is crucial for restoring the balance and developing effective therapeutics. Defined gut communities hold promise as next-generation probiotics, however, the challenge lies in selecting appropriate communities due to the high diversity of the gut microbiota, requiring strict parameters to ensure efficient restoration of dysbiotic environments. In this study, two minimal health-associated enterotype functional cores were assembled in vitro. These cores were based on core taxa found in each enterotype as well as health-association and functional capabilities. The communities were studied in vitro starting from monocultures grown in different carbon sources to gather information on substrate utilisation. Then to co-cultures and small communities were tested using different combinations of species to learn more about interactions within the community towards building the full community. The knowledge gathered both in silico in Chapter 3 and in vitro data from the monocultures on substrate use enabled to successfully design a suitable medium for the community. While results from the co-cultures experiments did not fully confirm in silico predictions in terms of competition or cooperations, the study highlighted key interacting taxa such as *Collinsella* or *Ruminoccocus*. Additionally, the study showed that four member communities built based on functional complementarity enabled members of the community to co-exist. Finally, the ~10 species community was subjected to perturbations by introducing different species of enterotype-driving genera either *Bacteroides* or *Prevotella*. The results indicated that the switching between species from the enterotype-driving genus group did not significantly affect the community composition under the tested conditions as time was the only significant variable that affected community profiles. Finally, the community showed to be resilient when tested with additional biotic and abiotic perturbations.

The study provides valuable insights into designing future biotherapeutics and contributes to a better understanding of community dynamics and interactions in the human gut microbiota, which is crucial for the design of efficient therapeutics.

Introduction

Many diseases have been linked to an imbalanced gut microbiota, referred to as dysbiosis, ranging from gastrointestinal disorders such as inflammatory bowel disease[63] to neurodegenerative disorders such as multiple sclerosis[67]. To restore the imbalance in the gut community, a better understanding of the community dynamics and interactions in the human gut microbiota is necessary. This will allow to not only facilitate the explanation of the aetiology of diseases, but also the design of efficient therapeutics. The use of defined gut communities as next-generation probiotics are promising therapeutics[84] however, designing and producing such communities remains a challenge.

The first challenge is the high diversity of the gut microbiota therefore, a defined community should be selected based on strict parameters to ensure that the chosen community can efficiently restore a dysbiotic environment. In previous studies, species were selected based on health, prevalence and abundance derived from 16S rRNA gene sequencing or metagenomics data from cohort studies[150,151,164]. However, gut communities have been shown to encompass different community types, referred to as enterotypes, within the population. As of now, four enterotypes have been described[14,16]: *Bacteroides* 1 (Bact1), *Bacteroides* 2 (Bact2), *Prevotella* (Prev) and *Ruminoccocus* (Rum). These enterotypes have also shown to have different functional profiles[152]. Therefore taking into account enterotypes as well as the functional core in addition to the taxonomic core within a population may be more biologically relevant in designing defined communities[56,58]. However, no studies have yet looked at cores within the different enterotypes.

Furthermore, studying defined communities in vitro will bring more insight into community interactions and dynamics[151]. While metagenomic sequencing can provide information on community functions and interactions, these tools only infer conclusions from highly complex dynamics encompassing hundreds of species. In vitro studies provide a simpler setting that allows for more confident links between microbial and metabolic readouts.

In this study, two minimal **health-associated enterotype functional cores** (M-HAEFC) were studied based on in silico selection of Bact1 and Prev enterotype core species as well as in silico analysis to determine their functional capabilities. By using different complexity levels of the communities (from co-cultures to 10 species communities), more knowledge

about species interactions was acquired. Finally, these M-HAEFCs were tested for their resilience with biotic and abiotic perturbation. Ultimately, the design of two M-HAEFCs based on health-association, functional capabilities and species composition will lead the way towards next-generation probiotics.

Methods

Minimal health-associated functional core selection

Health-associated enterotype functional core communities (HAEFC) were selected from the FGFP cohort[9] as described in Chapter 3. The selection was based on their prevalence and abundance in a healthy population, the presence of the different trophic levels as well as their identification as core members of the main three described enterotypes (e.g., Bact1, Prev and Rum). However, further analysis showed that a number of individual members of these communities exhibit high competition in silico as well as functional redundancy based on their functional capabilities. The in vitro research of these core communities will mainly focus on enterotypes Bact1 and Prev of which the cores are compositionally and functionally most distinct. Two **minimal health-associated enterotype functional cores** (M-HAEFC) were selected for the Bact1 and Prev enterotypes in order to avoid high competition within the community while still ensuring a fully functional profile. Several parameters were taken into account for the M-HAEFC species selection i.e. i) the average competition and cooperation indices of each species within the HAEFC community or closely related species (reverse ecology analysis), ii) the overall functional profile (i.e. unique functions that are not redundant in the M-HAEFC community, Supplementary Figure 1), iii) the available health-association profile (i.e. a literature review documenting possible associations of any of the selected species to diseases), iv) prevalence in each enterotype and v) belonging to a functional cluster. To this end, one species per genus or functionally related genus group (e.g *Faecalibacterium* / *Gemmiger* or *Agathobacter* / *Roseburia*) were kept for the M-HAEFC (Table 1, Supplementary Table S4.1).

Table 1. Minimal health-associated enterotype functional cores for enterotypes Bact1 and Prev*. In green, strains present and in black absent in either of the HAEFC.

Phylum	Strains	Community Bact1	Community Prev
Actinobacteria	*Collinsella aerofaciens* RCC1366	●	●
Bacteroidetes	*Alistipes shahii* RCC662	●	●
Bacteroidetes	*Phocaeicola vulgatus* RCC1806	●	●
Bacteroidetes	*Odoribacter splanchnicus* RCC828	●	●
Bacteroidetes	*Prevotella copri* DSM18205	●	●
Firmicutes	*Anaerostipes hadrus* DSM3319	●	●
Firmicutes	*Blautia obeum* DSM25238	●	●
Firmicutes	*Coprococcus comes* RCC3035	●	●
Firmicutes	*Dorea longicatena* DSM13814	●	●
Firmicutes	*Agathobacter rectalis* DSM17629	●	●
Firmicutes	*Faecalibacterium duncaniae* DSM17677	●	●
Firmicutes	*Ruminococcus bromii* RCC1377	●	●

*Overview of selection details is compiled in Supplementary Table S4.1.

Reverse ecology analysis

Reverse ecology analysis[159] was performed on the M-HAEFC species as described in Chapter 3. A reverse ecology approach is using the genomic features of species to infer the ecology of a community, for example interactions. Briefly, genomes were retrieved from IMG with their KO annotations. As described previously[159], this R package uses genome-scale metabolic network models in which functional annotated genomic data are used to reconstruct the metabolic network for each species. A set of seed metabolites is then used to identify which ones are specifically required by each species. Based on the seed set of each species, two interaction indices, competition and complementarity index are calculated for pair of species.

In vitro cultures

All cultures and preparations were performed at 37°C under anaerobic conditions in a Don Whitley A35 Anaerobic Workstation with HEPA filter (10% H_2, 10% CO_2, 80% N_2, 55% humidity). All media were preincubated overnight to remove oxygen before cultivation. The identity of the strains was regularly checked using 16S rDNA Sanger sequencing to ensure no contamination occurred. Monocultures were cultured either in RCM (Reinforced

Clostridial Medium (Oxoid, UK)): (*Anaerostipes hadrus* DSM3319, *Blautia obeum* DSM25238, *Coprococcus comes* RCC3035, *Dorea longicatena* DSM13814, *Agathobacter rectalis* DSM17629 and *Faecalibacterium duncaniae* DSM17677^T) or mGAM (modified Gifu Anaerobic Medium (Hyserve): (*Collinsella aerofaciens* RCC1366, *Alistipes shahii* RCC662, *Phocaeicola vulgatus* RCC1806, *Odoribacter splanchnicus* RCC828, *Prevotella copri* DSM18205, *Ruminococcus bromii* RCC1377, *Bacteroides uniformis* RCC1835, *Bacteroides thetaiotaomicron* RCC293A and *Prevotella lascolaii* RCC757). The medium used for co-cultures and communities in the study (MM++) contained 10g.L^{-1} of LB (Luria Bertani broth Sigma-Aldrich), 1.25g.L^{-1} sodium bicarbonate, 1g.L^{-1} xylan, 4 g.L^{-1} starch, 2 g.L^{-1} pectin, 0.2 g.L^{-1} glucose, 2 g.L^{-1} type II porcine mucin and 0.01 g.L^{-1} hemin dissolved in NaOH 0.05M. All the media recipes and details of the reagents can be found in Supplementary Table S4.2. The medium was autoclaved before the addition of hemin and adjusted to pH 6.0 after hemin addition unless stated otherwise. All fermentations were carried out anaerobically in Hungate tubes without pH control unless specified.

The tested panel of substrates were all tested in 10 g.L^{-1} of LB with 1.25g.L^{-1} of sodium bicarbonate and 1g.L^{-1} of the substrate: acetate (sodium acetate trihydrate, Sigma-Aldrich), butyrate (sodium butyrate Sigma-Aldrich), cellulose (Avicel® PH-101, Sigma-Aldrich) formate (sodium formate, Supelco), glucose (D-(+)-Glucose, Sigma-Aldrich), lactate (sodium DL-lactate solution 60% w/w Sigma-Aldrich), mucin (mucin from porcine stomach Type II Sigma-Aldrich), pectin (citrus pectin 57% methyl esterification, LFT-SMART, KU Leuven, Belgium), propionate (sodium propionate Sigma-Aldrich), starch (starch from potato (soluble), Sigma-Aldrich), succinate (sodium succinate dibasic hexahydrate Sigma-Aldrich), xylan (≥95 % xylooligosaccharides, from corncob, Carl Roth) or xylose (D-(+)- xylose Sigma-Aldrich).

Experiments not carried out in Hungate tubes were conducted in microbioreactors with automatic sampling and pH control using an Ambr® 15 Fermentation (Ambr® 15f) system (Sartorius Stedim Biotech, Royston, UK) inside a Don Whitley A155 Anaerobic Workstation with HEPA filter (10% H_2, 10% CO_2, 80% N_2, 55% humidity). The vessels were kept at 37°C (± 0.3°C) by individual vessel heaters, while each vessel was stirred at 300 rpm by a single Rushton-like impeller. The pH was measured using optical sensor patches, with base (1 M NaOH) automatically added at a target pH of 6.3 and a trigger pH

of 6.0 if needed. The pH was also measured offline using an analysis module which automatically re-calibrated reactor sensors when necessary.

Co-cultures were grown in transwell plates with a pore size membrane of 0.2 μm. 900 mL of medium (MM++) was added on each side of the membrane and 100 μL each isolate was added from overnight grown cultures on each side. The cultures were performed in duplicates and were incubated for 24 hours before measuring cell count with flow cytometry.

Community assembly and sampling

For in vitro assembly of M-HAEFC communities, cells were inoculated at a concentration of approximately $5x10^6$ cells per mL. The number of cells for each inoculation was determined by flow cytometry, after which the cells were diluted in the MM++ medium and mixed in even ratios. 1 mL of each community inoculum was used to inoculate the Hungate tubes or microbioreactors to a total volume of 10 mL in triplicates. After 16 hours of incubation, 1 mL was sampled for storage at -80°C, and 1 mL of fresh medium was added in either Hungate tubes or Ambr vessels. During Ambr® 15f fermentations, at 24 and 32 hours, 200 μL samples was pipetted into a cooled plate (4°C) and kept until the end of the experiment.

For each sample (tube or Ambr® 15f), 50 μL of the samples were used for cell count by flow cytometry directly. The cells of the remaining sample were pelleted by centrifugation at 3220 RCF for 10 minutes. Both pellet and supernatant plates were stored separately at −80°C until they were used for community sequencing and HPLC, respectively.

Flow cytometry

For assembly of co-cultures and communities, monoculture cell count was determined by flow cytometry after 18 hours of growth. Next, cell suspensions were serially diluted 5000× in PBS to an approximate cell density of 10^6 cells per mL and stained with 1 $\mu l.mL^{-1}$ SYBR green I (1:100 dilution in dimethylsulfoxide; 20 min incubation at 37°C; 10.000 concentrate, Thermo Fisher Scientific) as previously described[16]. Samples were measured with a benchtop CytoFLEX S flow cytometer (Beckman Coulter) which was calibrated daily with CytoFLEX Daily QC Fluorospheres.

Community composition analysis

Long read sequencing with Nanopore (Oxford Nanopore Technologies)

Complex communities (M-HAEFC) were pelleted after fermentation to be subjected to DNA extraction using the PowerMicrobiome DNA/RNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, USA) according to the manufacturer's instructions as described previously[9]. 9uL of the DNA extract was taken and used for library preparation with the Rapid Barcoding Kit 96 (SQK-RBK110.96, Oxford Nanopore Technologies ONT). Nanopore sequencing was performed at room temperature using R9.4.1 flow cells and a MinION Mk1B device following manufacturer's instructions with a run-time set at 24 hours. Basecalling of reads was performed with guppy v6.1.1 (ONT) in super high accuracy mode, then barcodes and adaptor sequences were trimmed from basecalled reads. Basecalled reads were quality filtered using the default Q-score of 9 and reads below this threshold were discarded. Taxonomic profiling was performed with Kraken2[165] v2.0.9 using a custom database constructed from genomes of the Genome Taxonomy Database (GTDB) release r202[135,166] (Supplementary Table S4.3). This custom database comprises publicly available genomes of selected strains, wherever feasible. In instances where genomes of selected species were not available, all genomes from the database were utilised for the corresponding species, specifically for the RCC strains. Kraken2 database construction was performed using Struo2[167] v2.2.2 with genomes downloaded from genbank4 with the default k-mer length (35) and default l-mer length (31). Taxonomic calls were made using the default minimum hit group (minimum overlapping k-mers sharing the same minimiser) threshold of 2.

Cellscanner

Samples that did not yield sufficient read number with ONT, which was typically the case for four-member communities, were subjected to Cellscanner for community composition analysis. This tool is using flow cytometry data and machine learning clustering to identify members of a community and was used to assess community composition in co-cultures with up to four members as described previously[168,169]. Briefly, CellScanner relies on flow cytometry data from monocultures which are used as reference files to train 10 classifiers (i.e., neural networks of 200 layers, using lbfgs solver and the rectified linear unit activation function). For each classifier, 1,000 events per species are selected randomly from the

corresponding monoculture. Of these, 875 events (7/8) are used to train the neural network and 125 events (1/8) to test the trained neural network. Each trained classifier then assigns a species to each event in the co-culture. This method is high throughput for low diversity samples and requires very little sample processing.

Metabolic readouts

For metabolic analysis, frozen supernatant samples were thawed from −80°C (30 minutes, room temperature). Concentrations of glucose, ethanol, succinic acid, lactic acid, formic acid, acetic acid, propionic acid, and butyric acid were measured using an Agilent 1200 HPLC (Diegem, Belgium) system equipped with an Aminex HPX-87 H column (Bio-rad, Temse, Belgium). The column was kept at 40°C and eluted with 5 mM H_2SO_4 at a rate of 0.60 $mL.min^{-1}$. All targeted compounds were detected with refractive index changes (Agilent RID, at 40°C).

Perturbation with E. coli and ampicillin

The B1 M-HAEFC was assembled and grown for 16 hours anaerobically in the Ambr® 15f system. Each vessel was filled with MM++ at pH 6.0. At 16 hours after inoculation, 10% of the fermentation liquid was replaced by either i) fresh medium, ii) fresh medium with *E. coli* DSM18039, iii) fresh medium with *E. coli* DSM18039 and 100 $\mu g.mL^{-1}$ of ampicillin or iv) fresh medium with 100 $\mu g.mL^{-1}$ of ampicillin. *E. coli* DSM18039 was grown overnight in mGAM and diluted to an OD_{600} of 0.8 before being added in a 1/100 dilution into the vessels. The monocultures were grown for 24 hours with different concentrations of ampicillin (10 $\mu g.mL^{-1}$, 50 $\mu g.mL^{-1}$ and 100 $\mu g.mL^{-1}$) and without ampicillin in their preferred medium (mGAM or RCM), OD_{600} was measured to assess growth of each isolate in duplicates. Their growth without ampicillin was subtracted to their growth in ampicillin to assess their growth potential in the presence of the antibiotic.

pH perturbation

Perturbations at pH 5.0, 6.0 and 7.0 were performed in triplicate using the Ambr® 15f system. The MM++ medium was adjusted to a specific pH before inoculation. Four hours after inoculation, and after 8, 16, 24 and 32 hours, 120 µL of 1M NaOH was added to each

reactor. After 16 hours, 10% of the volume was changed to fresh medium with the respective pH to compensate for the liquid lost by sampling and to renew nutrients.

Statistics

Graphical representations were performed in either R using ggplot2[140] or JMP®(Version 17, SAS Institute Inc., Cary, NC, 1989–2021). The contribution of metadata variables to the community or metabolomic variation was determined by dbRDA on genus-level Aitchison distance (Bray-Curtis distances between samples) or Euclidean distances respectively with the capscale function in the vegan R package[138]. Correction for multiple testing (Benjamin–Hochberg procedure, FDR) was applied and significance was defined at FDR < 0.1. The cumulative contribution of metadata variables was determined by forward model selection on dbRDA with the ordiR2step function in vegan[138], with variables that showed a significant contribution to community variation in the previous step. All other statistical analyses were performed using JMP® (Version 17, SAS Institute Inc., Cary, NC, 1989–2021).

Results

Designing a culture medium for the M-HAEFC

To assemble a defined community that was designed in silico, a culture medium had to be designed in such a way that it would support the growth and co-existence of all species. The metabolic potential of each species was characterised in silico to formulate a medium containing all substrates required for growth of each member in the community. Next, these in silico predictions were evaluated under in vitro test conditions (Supplementary Figure 1). For this purpose, the metabolic ID initiated in Chapter 3 based on in silico analysis was expanded to include in vitro analyses. First, the metabolic readouts of all M-HAEFC monocultures were assessed by HPLC when grown in their preferred medium (mGAM or RCM) (Figure 1 A). As expected, known butyrate producers such as *Faecalibacterium duncaniae* produced butyrate in higher concentrations than other species (>20mM). In the tested conditions, lactate was only produced in large quantities by *Phocaeicola vulgatus* while succinate was produced in small amounts mostly by Bacteroidetes taxa. Due to technical issues, propionate measurements were not obtained in this experiment.

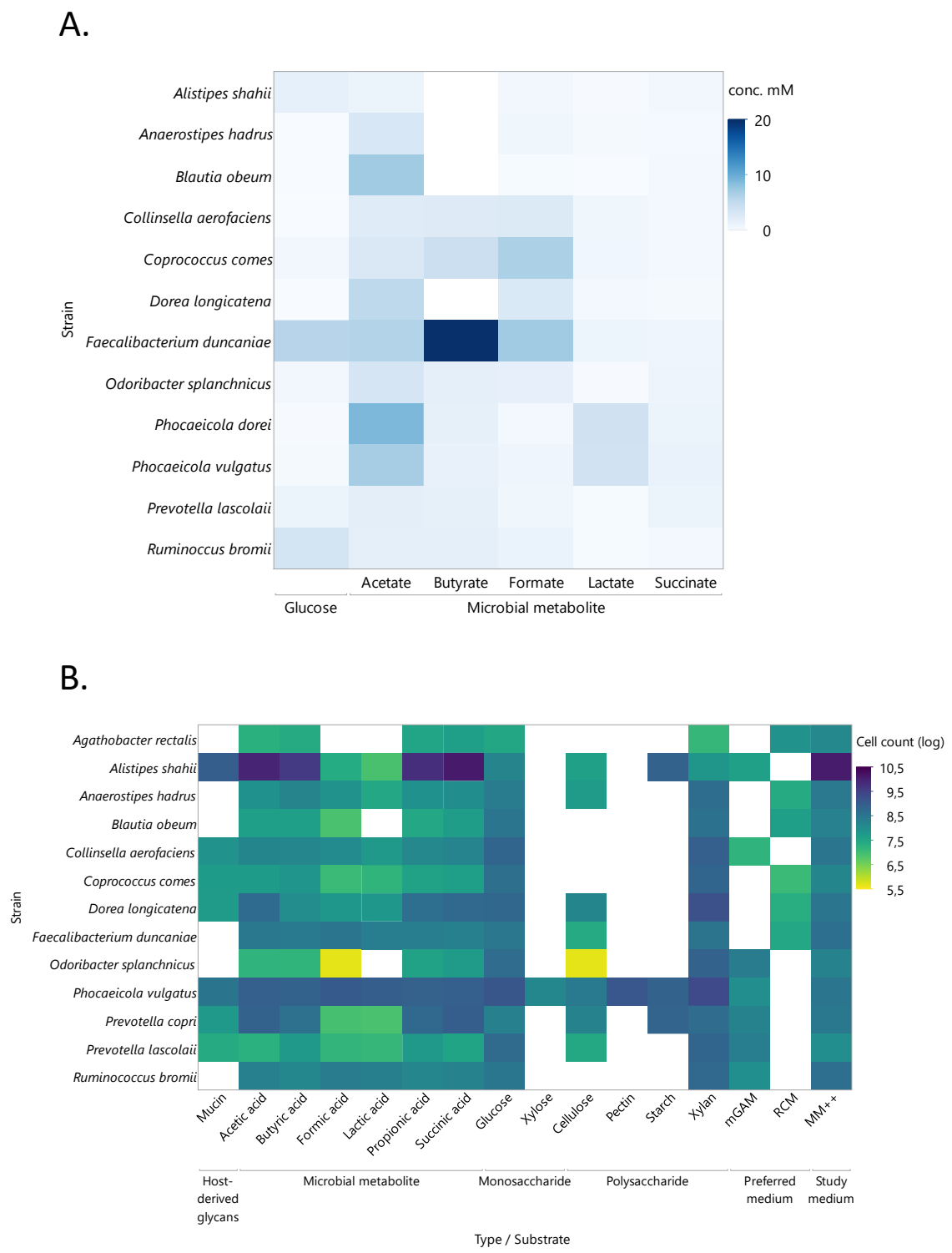


Figure 1. A. Average metabolic readouts of monocultures grown on their preferred medium (either mGAM or RCM) in triplicates. B. Log-scale cell count reached by each strain-substrate combination. 1 g.L^{-1} of each substrate was added to LB medium. No growth was observed where there is a white square. The right panel depicts growth results in MM++ medium containing a combination of complex substrates (mucin, pectin, starch and xylan). The preferred medium refers to the commercial medium (either mGAM or RCM) that each species typically grows on and is used as a reference.

Next, in vitro substrate preferences were assessed for each strain (Figure 1 B). Substrates in the test panel were selected based on the outcome of the initial in silico analyses and were now tested under specific in vitro conditions. To ensure that each species would only utilise the tested substrate, the substrates were added to LB, a medium that did not contain any carbohydrate sources. Most of the species were capable of growing in the presence of all microbial metabolites, glucose and xylan. Only a limited set of species could use starch (i.e., *Prevotella copri, Pho. vulgatus* and *Alistipes shahii*) or pectin (*Pho. vulgatus*) as a substrate while most species could grow on mucin (7/13) or cellulose (8/13).

After finalising the metabolic ID through in vitro analysis, the next step was to design the culture medium enabling growth of all M-HAEFC members. A medium composed of multiple complex carbohydrates was envisaged to allow specialists to utilise their preferred complex carbon source while other species could rely on metabolic cross-feeding for growth. Therefore, mucin, pectin and starch were chosen as they were substrates that only a few species could grow on (Figure 1 B). Xylan was the final complex compound added as all species showed to grow on this substrate to ensure that each species would have a complex carbon source to utilise. Xylan is a hemicellulose and compared to cellulose, xylan contains many different side chains, making it a more complex carbon source. For this reason, cellulose was not included in the medium as only a few species could grow on it. A small amount of glucose was added to the medium to allow slower growers to start their growth before cross-feeding on intermediate products of other species. Finally, no microbial metabolite such as acetate or succinate were added to the medium to avoid reducing cross-feeding interactions. Therefore, the final medium (MM++) used in this study contained: LB (as source of nitrogen, vitamins and salt), bicarbonate (acting as a physiologically relevant buffer[170]), xylan, starch, pectin, glucose, mucin and hemin.

Cooperation and competition in M-HAEFCs: insights from in silico and in vitro experiments

To ensure low competition and high cooperation within the two M-HAEFC communities (Bact1 and Prev), reverse ecology analysis was performed on the selected community.

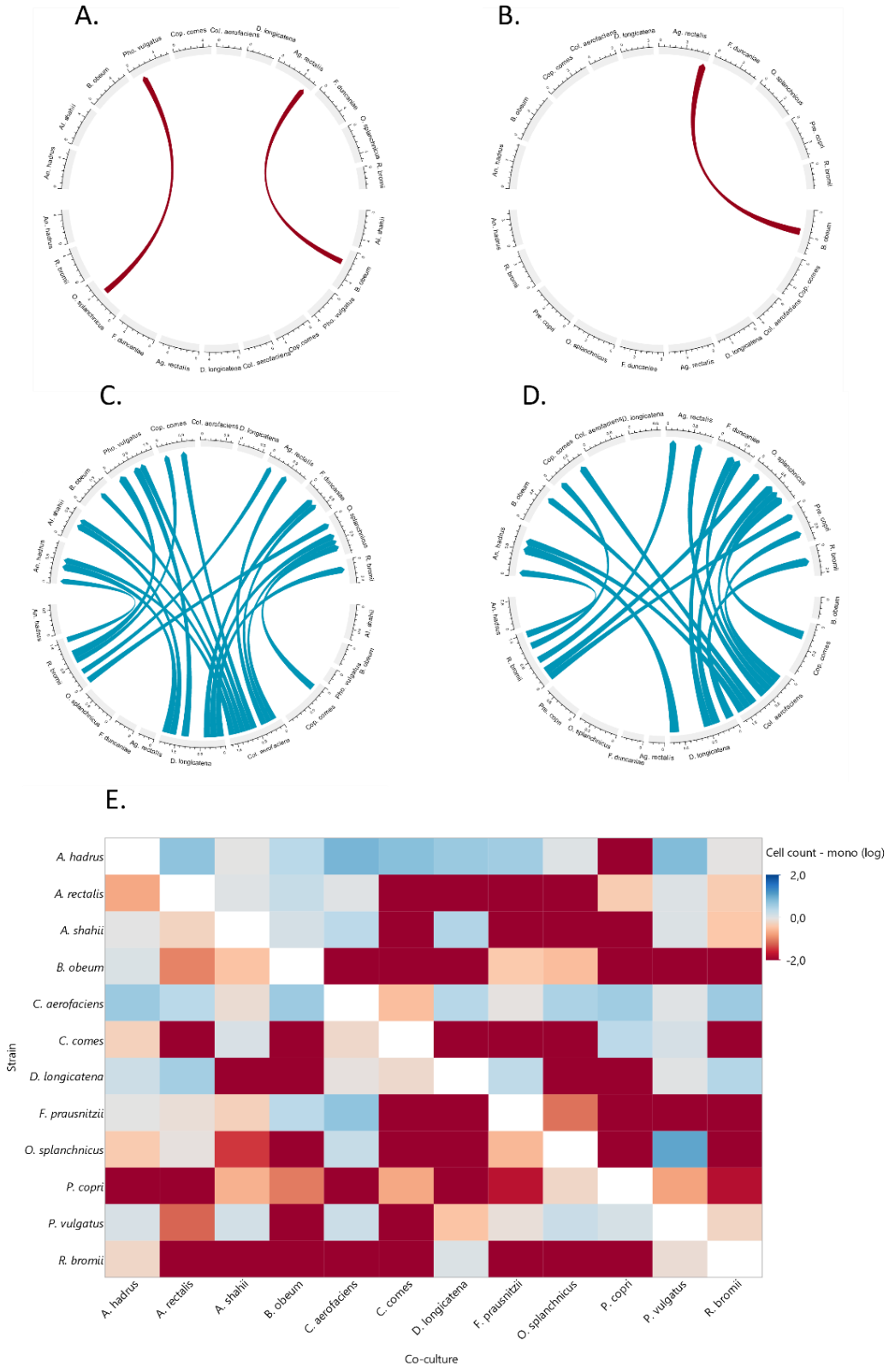


Figure 2. In silico analysis using reverse ecology analysis[159] depicting competition with an index >0.75 (A & B) and cooperation with an index >0.20 (C & D) present in communities containing Bact1 (left, A and C) and Prev (right, B and D) species. E. Cell count results of in vitro co-culture transwell results for all strains in the Bact1 and Prev communities showing growth (cell count $\log_{10}$) subtracted to monoculture for all combinations.

In silico analysis revealed only two competitive pairs of species with an index higher than 75% (Figure 2 A and B respectively). In the Bact1 M-HAEFC, *Odoribacter splanchnicus* was predicted to compete with *Pho. vulgatus*. These species were found in the same functional cluster in the in silico functional analysis (Supplementary Figure S3.1), suggesting competition for one or more substrates essential for growth. In the Prev M-HAEFC, competition was predicted between *Blautia obeum* and *Agathobacter rectalis*. On the other hand, both M-HAEFC assemblies also showed a high number of cooperations with an index higher than 20% (n= 20 for Bact 1 and n= 17 for Prev; Figure 2 C and D respectively). In both communities, *Dorea longicatena*, *Ruminococcus bromii,* as well as *Collinsella aerofaciens* showed a high cooperation index (n = 6/4, n = 5/5, n = 8/7, respectively, in Bact1/Prev).

Towards experimental validation of these in silico predicted competition/cooperation pairs, co-cultures were performed in transwells separated by a 0.2µm membrane to avoid direct cell-cell contact between combined strains. The transwell setup still allowed strains to cooperate or compete for resources in the shared medium. Using this system, many competitions or cooperations among the different species were observed (Figure 2 E and F; Supplementary Table S4.3), these interactions were defined with a growth difference of 0.5 log compared to the monoculture growth (either negative, competition, or positive, cooperation). The results showed that *Ag. rectalis, Pho. vulgatus* and *Col. aerofaciens* showed to be more cooperative with the rest of the community. However, overall, the number of cooperations observed was less important compared to the in silico analysis and species such as *D. longicatena* with high predicted cooperation in silico (cooperation with about 50% of species with a cooperative index >20%) and forming a cooperative hub, was not particularly more cooperative in the tested conditions. Conversely, the in vitro results about competition (where growth in co-culture was less than 0.5 log difference compared to the monocultures), showed that three species *Cop. Comes, Pre. copri* and *O. splanchnicus,* did not favour the growth of more than half of the species (66.7%, 58.3%, and 58.3% respectively). These competitions were not predicted in silico, which may highlight the limitations of in silico predictions as interactions highly depend on the environment e.g., the available substrates (medium). Moreover, the fact that some specific interactions might be strain-dependent could explain the difference between the in silico and in vitro results.

Table 2. Number of cooperative partners in silico (only including cooperative indices > 20%) and in vitro (with co-culture with log growth > 0.5 compared to monocultures), in absolute numbers (left column) and percentages (right column).

	Bact 1				**Prev**			
	In silico (coop > 20%)		In vitro (log_{10} growth >0.5)		In silico (coop > 20%)		In vitro (log_{10} growth >0.5)	
An. hadrus	0	0.0%	1	9.1%	0	0.0%	1	10.0%
Ag. rectalis	0	0.0%	2	18.2%	0	0.0%	2	20.0%
Al. shahii	0	0.0%	0	0.0%				
B. obeum	0	0.0%	1	9.1%	0	0.0%	1	10.0%
Pho. vulgatus	0	0.0%	2	18.2%				
Col. aerofaciens	8	72.7%	2	18.2%	7	70.0%	2	20.0%
Cop. comes	1	9.1%	1	9.1%	1	10.0%	1	10.0%
D. longicatena	6	54.5%	1	9.1%	4	40.0%	1	10.0%
F. duncaniae	0	0.0%	1	9.1%	0	0.0%	1	10.0%
O. splanchnicus	0	0.0%	1	9.1%	0	0.0%	1	10.0%
Pre. copri					0	0.0%	1	10.0%
R. bromii	5	45.5%	1	9.1%	5	50.0%	1	10.0%

Next, 4-member communities were designed with members of both M-HAEFC communities to explore if any competition would result in the exclusion of a species. These minimal communities were designed using trophic levels by taking into account the functional clusters from Chapter 3 by including one member of cluster 1, 2 and 3 (Figure 3 A; Supplementary Table S4.4). Additionally, *Col. aerofaciens* and *R. bromii* were added to the communities as two species not belonging to any cluster. Two communities were selected for both Bact1-like and Prev-like communities which were fermented for 24 hours in MM++ and community and metabolic profiles were analysed at the end of the fermentation. CellScanner was used to analyse the data with an average training accuracy of 0.40 for the four communities (average of duplicates; B1 =0.40, B2 =0.42, P1=0.39, P2 =0.40), and the following average sensitivity and precision respectively per species: *Pho. vulgatus* (0.51, 0.53), *B. obeum* (0.37, 0.38), *C. aerofaciens* (0.33, 0.42), *F. duncaniae* (0.38, 0.37), *P. lascolaii* (0.57, 0.40), *R. bromii* (0.37, 0.39). Fifty-one per cent of unknowns were found on average in the four communities. The low accuracy can be in part explained by the low number of all cells used in the prediction (n=448).

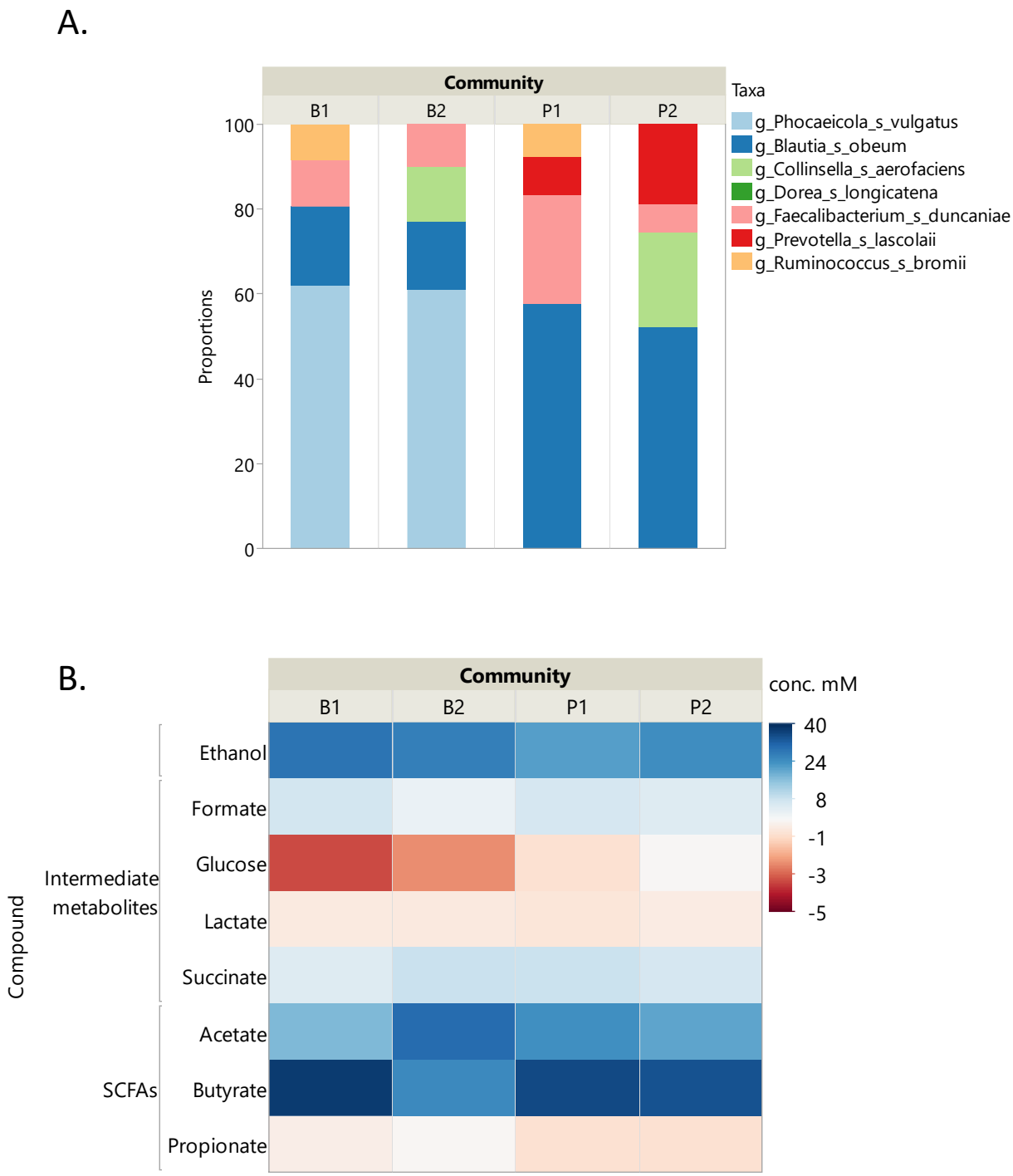


Figure 3. A. Community composition 24 hours after fermentation analysed by CellScanner for two Bact1 small communities (B1 and B2) and two Prev small communities (P1 and P2). C. Average metabolite production of each community at 24 hours after fermentation in duplicates measured by HPLC.

The 4-member communities showed that all species in each combination could co-exist without exclusion during the entire fermentation experiment (Figure 3 A), with a high dominance of *Pho. vulgatus* in the Bact1-like communities and of *B. obeum* in the Prev-like communities. Ethanol, acetate, succinate and butyrate were produced in all communities, while glucose, lactate and propionate were consumed. While the MM++ medium contains a limited amount of glucose (0.2 $g.L^{-1}$), a negative glucose concentration indicates a consumption, while a positive concentration compared to the blank medium indicates either limited consumption from the medium or release of glucose in the medium.

Testing the resilience of the two M-HAEFCs

In the final part of the study, the two M-HAEFC were assembled as full communities to include all selected members. In parallel, the resilience of these communities was tested by introducing different species of the enterotype-driving genus group, i.e., *Bacteroides/Phocaeicola* or *Prevotella*. The setup comprised the original full community M1 or M5 in parallel with modified communities M2/M3 and M7, fermented for 24 hours in MM++ at pH 6.0. After 16 hours, the 10% of the fermentation was replaced with fresh medium.

For the Bact1-like community, *Pho. vulgatus* (M1) was replaced by *B. uniformis* (M2) or *B. thetaiotaomicron(*M3), two other highly prevalent species in this enterotype with similar functional profiles (Figure 4 A). Although *B. thetaiotaomicron* is also part of the healthy core microbiota, unlike *Pho. vulgatus, Pho.dorei* and *B. uniformis,* it was not found in the Bact1 core. However, given that *Pho. vulgatus* and *Pho. dorei* are phylogenetically closely related, *B. uniformis* was chosen along with *B. thetaiotaomicron* to investigate if the change of species would lead to differences in the community composition.

The effect size analysis of covariates indicated that the community, from the different *Bacteroides/Phocaeicola* spp. accounted for a significant proportion of the variation observed between samples (36%), while time point accounted for an additional 40% (total of 76%, dbRDA; Figure 4 B and C). In contrast, replicates did not contribute significantly. In this experiment, all members but *Anaerostipes hadrus* were detected in the communities.

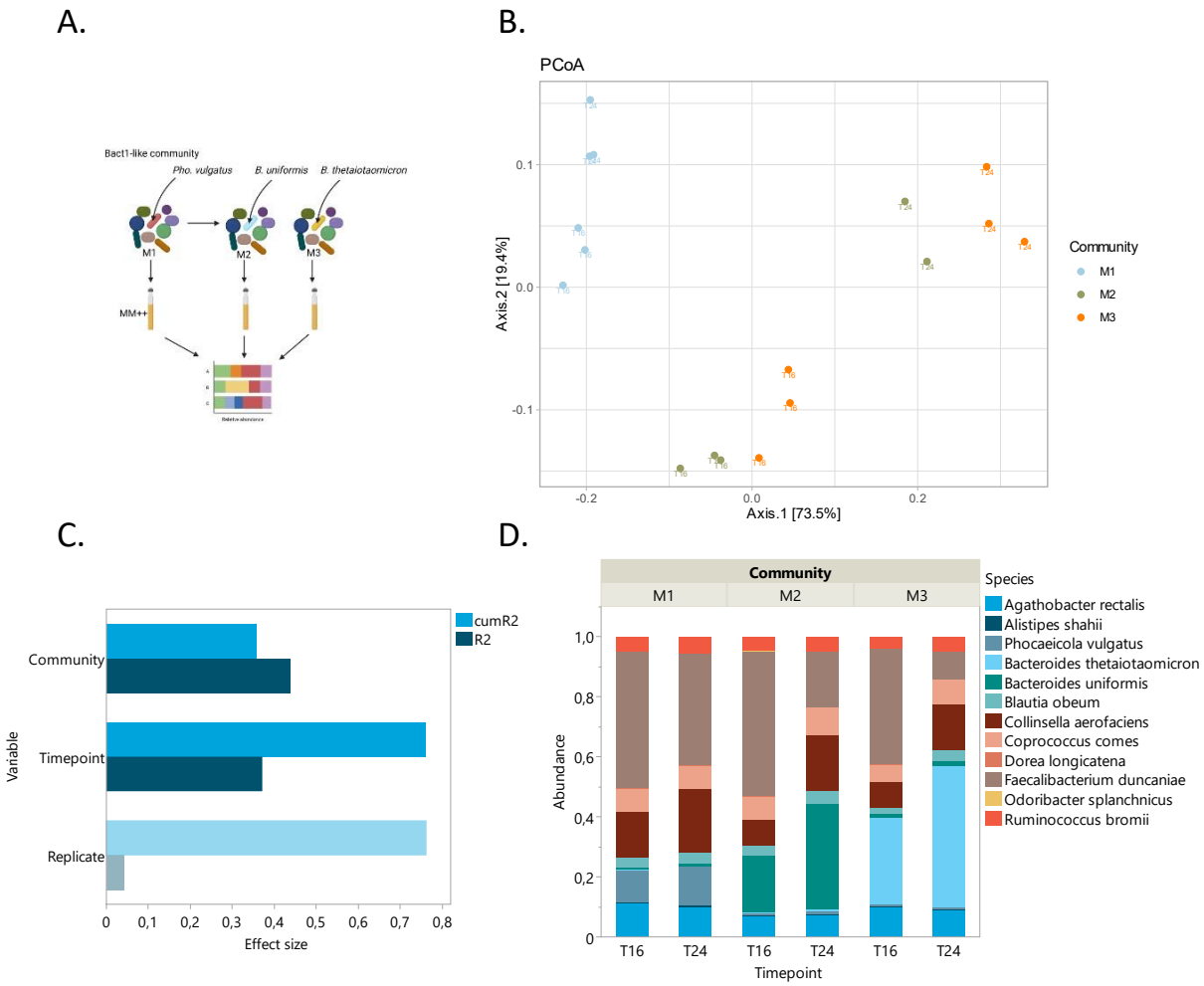


Figure 4. A. Experimental set-up of full communities: the original Bact1-like community (M1) was modified by replacing *Pho. vulgatus* by either *B. uniformis* (community M2) or *B. thetaiotaomicron* (community M3). Each community was then cultured for 24 hours in MM++ at pH 6.0. The community compositions were then compared at 16 and 24 hours. Image made with Biorender. B. Genus-level community variation of the fermentations in triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different communities (M1, M2 and M3, colours). C. Variables contributing most to compositional variation in the fermentations (dbRDA, species level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes), the not significant variable is in lighter colour $p > 0.05$. D. Community composition at species level with *Pho. vulgatus* (M1), *B. uniformis* (M2) *and B. thetaiotaomicron* (M3) at 16 and 24 hours.

While the community (M1, M2, M3) accounted for most of the variation, overall, at 16 hours the three communities had similar community composition with a high abundance of *F. duncaniae.* At 24 hours, *B. uniformis* and *B. thetaiotaomicron* dominated the communities (M2 and M3) to a greater extent, with a relative abundance of 0.4 and 0.5 respectively, compared to *Pho. vulgatus* with a relative abundance of 0.1 (M1) (Figure 4 D) and *F. duncaniae* was decreased in all communities (Kruskal-Wallis with post hoc Dunn test $p < 0.002$). This effect could be due to the relative abundance of *Phocaeicola/Bacteroides* increasing overtime, here absolute quantification of the communities would be necessary to have a better understanding of the dynamics. However, despite differences in relative abundances among the three communities, all three allowed the entire community to coexist, indicating that the presence of *Bacteroides/Phocaeicola* species had a limited effect on the overall community composition.

For the Prev-like community, two species were selected, i.e., the highly prevalent and abundant species *Pre. copri* and the low abundant *Pre. lascolaii* (Figure 5 A). Here, no variable significantly explained any differences observed between the communities, which may be attributed to the low number of samples (n=6, data not shown). Nonetheless, upon examining the community composition at both 16 and 24 hours, the communities had a similar composition, with a high dominance of *Prevotella* spp. and *F. duncaniae* accounting in average for 80 and 90% of the total relative abundance in M5 and M7 respectively. Here also, all taxa were detected in both communities (Figure 5 B). These results suggest that these two species occupy a similar niche and therefore either species can be used to design a stable Prev-like community.

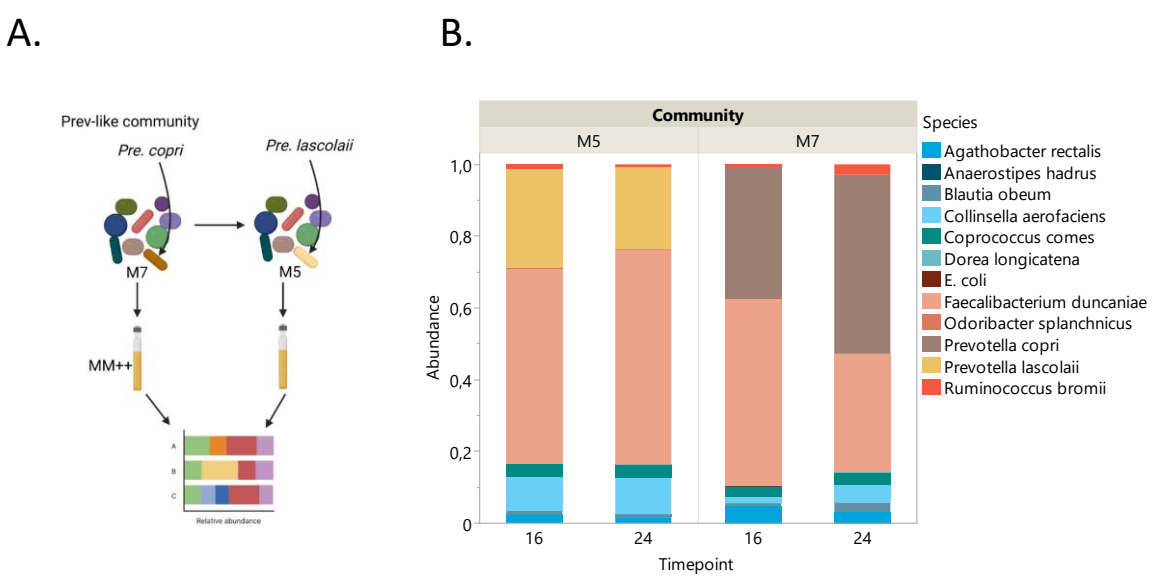


Figure 5. A. Experimental set-up of full communities: the original Prev-like community (M7) was modified by replacing *Pre. copri* by *Pre. lascolaii* (community M5). Each community was then cultured for 24 hours in MM++ pH 6.0. The community compositions were then compared at 16 and 24 hours. Image made with Biorender. B. Community composition with *Pre. lascolaii* (M5) and *Pre. copri* (M7) at 16 and 24 hours.

To further test the resilience with a biotic and abiotic stressor on the Bact1-like community (M1), a broad range antibiotic (ampicillin) and a potential pathobiont (*E. coli* DSM18039) were added after 16 hours to the Bact1 M-HAEFC community (Figure 6 A). As *E. coli* is a fast grower, it could overtake an unstable community once administered. Different antibiotics were initially tested on monocultures to test if individual species would be affected by different antibiotics (data not shown). The tested antibiotics encompassed different modes of action including inhibition of protein synthesis (erythromycin and chloramphenicol), inhibition of cell-wall formation (ampicillin) and binding to bacterial ribosomes (kanamycin). Overall, all species showed a lower growth in their preferred medium plus ampicillin compared to their growth in their preferred medium only (RCM or mGAM, Figure 6 B). Therefore, ampicillin showed a negative effect on these strains compared to other antibiotics tested and was kept to test the community resilience (Figure 6 B, data not shown).

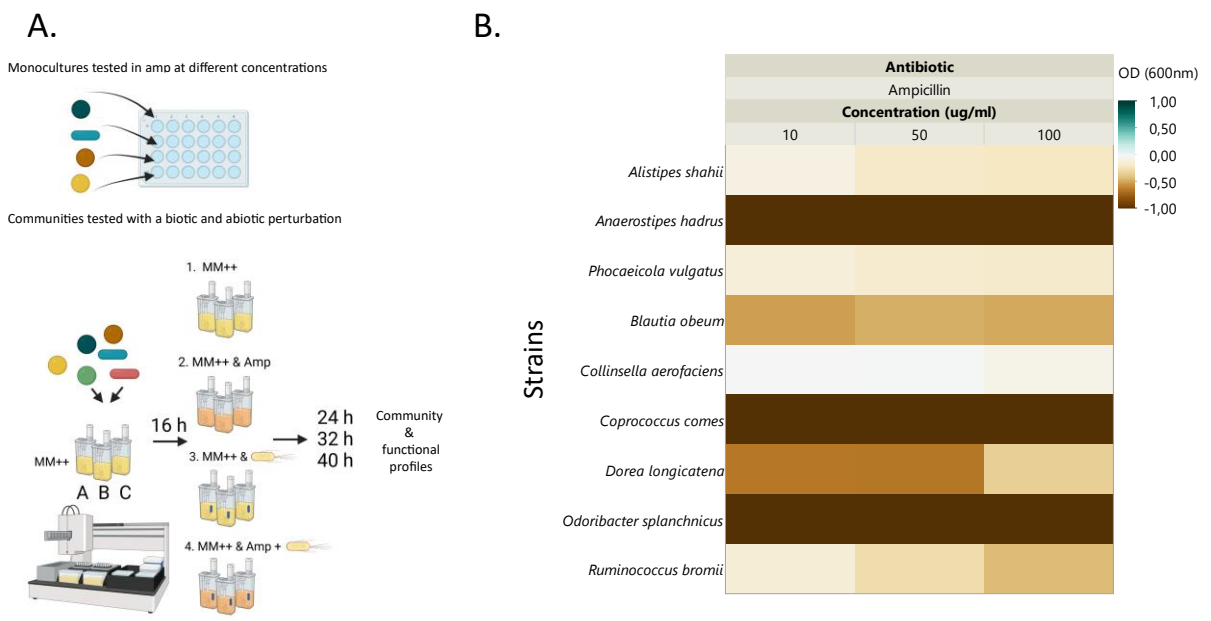


Figure 6. A. Experimental design: the growth of each isolate was tested with different concentrations of ampicillin compared to their preferred medium (mGAM or RCM). Then, the M-HAEFC community (M1) was grown in triplicates for 16 hours and 10% of the medium was replaced with either fresh medium only (MM++, pH 6.0), fresh medium with 100 μg.mL^{-1} of ampicillin, fresh medium and *E. coli* or fresh medium with 100 μg.mL^{-1} of ampicillin and *E. coli*. The community was then further cultured until 40 hours, samples were taken every 8 hours and another 10% of the medium was replaced at 32 hours. The community was kept at a constant pH during the experiment using the Ambr® 15f platform. Image made with Biorender. B. Growth of each isolate (OD_{600}) in different concentrations of ampicillin (10 μg.mL^{-1}, 50 μg.mL^{-1} and 100 μg.mL^{-1}) subtracted to their growth in medium only in duplicates (RCM or mGAM).

The results showed that although *E. coli* engrafted in the community with and without the addition of antibiotics after 32 hours (in red in Figure 7 C), only time had a significant effect on community composition (dbRDA effect size = 26%, Figure 7 A and B). Replicate and perturbation were not significant variables, indicating that neither ampicillin nor *E. coli* affected the community under the tested conditions. The replicates showed a significant univariate effect likely due to the low number of samples in the experiment which amplifies this effect, one replicate showed a higher relative abundance of *Faecalibacterium prausnitzii* already at 16 hours (Supplementary Figure S4.1). However, the replicates didn't show a significant effect size at 16 hours, hence before the perturbation (RDA $p > 0.05$). This result shows that the experimental set-up did not introduce additional variation.

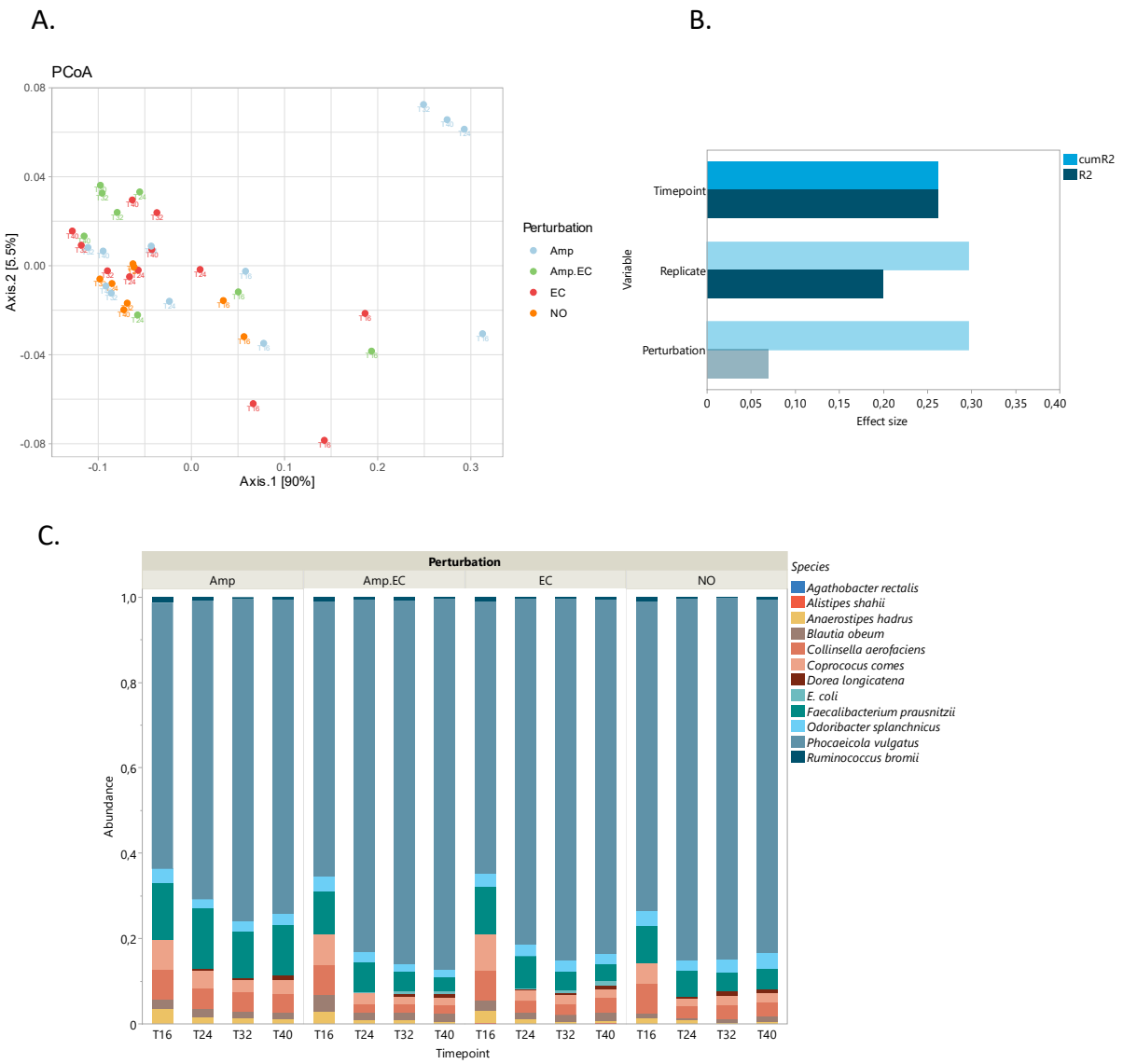


Figure 7. A. Species-level community variation of the fermentations in triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different perturbations (colours). B. Variables contributing most to compositional variation in the fermentations (dbRDA, species level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes), not significant data in light colours $p > 0.05$. C. Community composition at species level over time per perturbation (average composition of the triplicates).

Regarding the metabolic profile, time was also the only significant variable and the accumulation of metabolites overtime in all perturbations (Figure 8 A, B and C, dbRDA = 24%). Perturbation and replicates were not significant variables. Time had a significant effect for all metabolites except for formate and ethanol. Acetate, butyrate, propionate and succinate significantly increased from 16 hours to 32 and 40 hours but not between other

timepoints (Kruskal-Wallis $p < 0.001$, Figure 8 D), which suggest that the community functional profile is stabilising after 24 hours (32 hours for propionate). Conversely, glucose and lactate were only detected at 16 hours and were significantly decreased at subsequent timepoints, suggesting that the community consumed these metabolites. These results indicate that each community lead to accumulation of propionate, acetate, butyrate and succinate while lactate and glucose were consumed.

These results show that in the tested conditions, the perturbation did not significantly alter community composition and metabolic profiles therefore indicating that the community was resilient in the tested conditions.

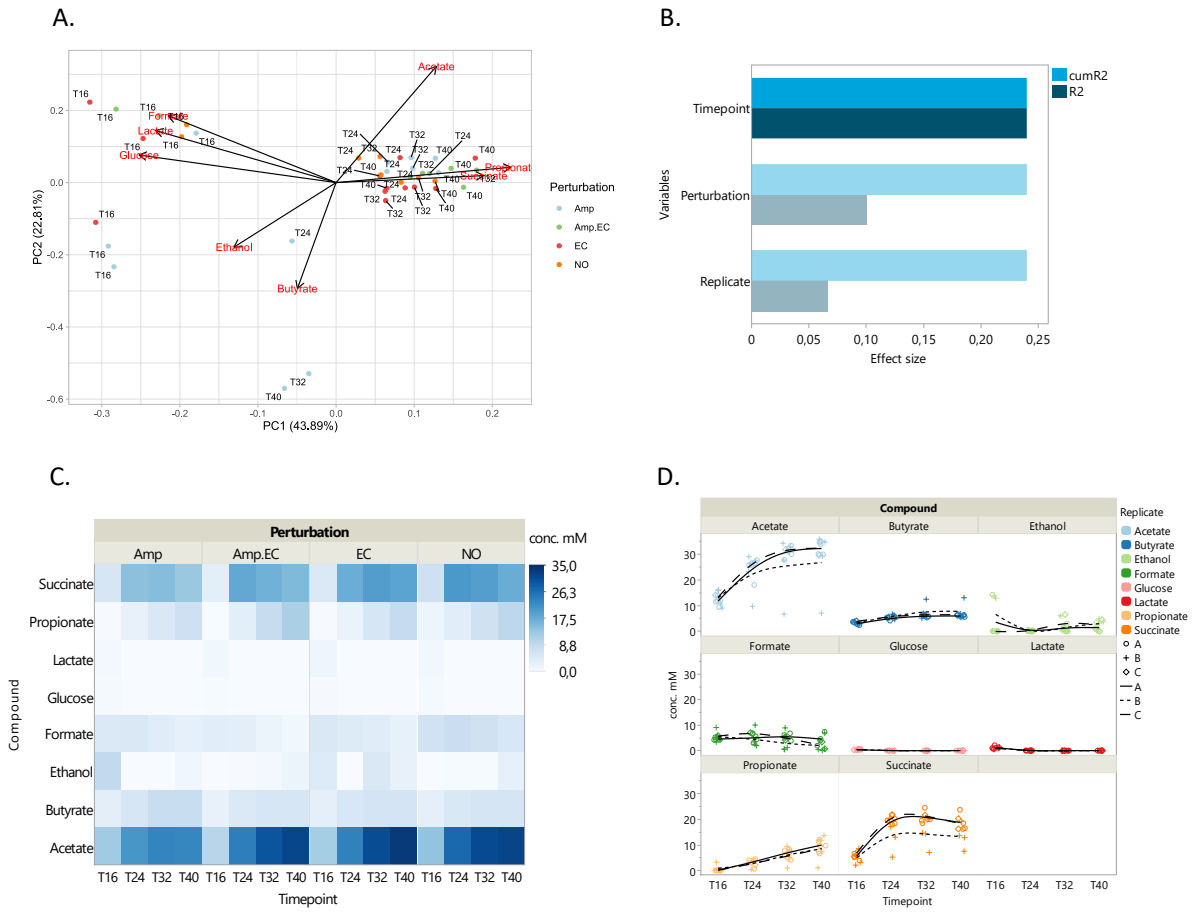


Figure 8. A. Metabolite composition variation of the fermentations in triplicates, represented by principal coordinates analysis (Euclidean distances, PCA). Shown are samples from the different perturbations (colours). B. Variables contributing most to metabolic profile variation in the fermentations (dbRDA, Euclidean distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes) not significant data in light colours $p > 0.05$. C. Average metabolic profiles for each community over time (in triplicates) D. Concentration of each metabolite (mM) over time in all the samples with replicates shown.

Impact of pH on community composition and function

In addition to nutritional content, also pH of the culture medium may impact growth of monocultures and artificial community assemblies[171,172]. Within the colonic pH range of 5.2-7.0[6], the starting pH in a parallel fermentation series was set at pH 5.0, 6.0 or 7.0 to evaluate effects on community composition and function.

Both pH and time significantly affected community composition and function (dbRDA effect size = 54 and 26%, with a combined effect size of 77% for the Bray-Curtis distances (composition), and effect size = 42% and 38%, with a combined effect size of 76% for the Euclidean distances (function) (Figure 9 B and C, Figure 10 A and B). The replicates were not significant for community composition and function. The taxa present in each community was overall similar except for *R. bromii* which had an average relative abundance of 0.4% at pH 5.0 while it was not always detected at other pH (not detected in 8 samples out of 18, with an average of 0.1% relative abundance). In contrast, *Pho. vulgatus* dominated the community at pH 6.0 and 7.0 from 16 hours while at pH 5.0 only at 40 hours.

All species but *O. splanchnicus* had a significant change in relative abundance in different pH (Kruskal-Wallis post hoc Dunn test $p < 0.05$), most between pH 5.0 and 7.0 except for *An. hadrus* and *B. obeum* also between pH 5.0 and 6.0. Therefore, we tested the growth of two species (*F. duncaniae* and *An. hadrus*) in monocultures at different pH (Figure 9 E). *F. duncaniae* was significantly increased in the communities in pH 5.0 compared to 7.0 (Kruskal-Wallis post hoc Dunn test $p < 0.001$), while in monocultures it was increased in pH 6.0 (only significant difference between pH 6.0 and 7.0 (Kruskal-Wallis post hoc Dunn test $p < 0.03$)). *An. hadrus* was significantly increase in the communities in pH 5.0 compared to pH 6.0 and 7.0 (Kruskal-Wallis post hoc Dunn test $p < 0.05$), which was reflected in monocultures with also a significant increase in pH 5.0 (significant difference between pH 5.0 and both 6.0 and 7.0 (Kruskal-Wallis post hoc Dunn test $p < 0.001$)).

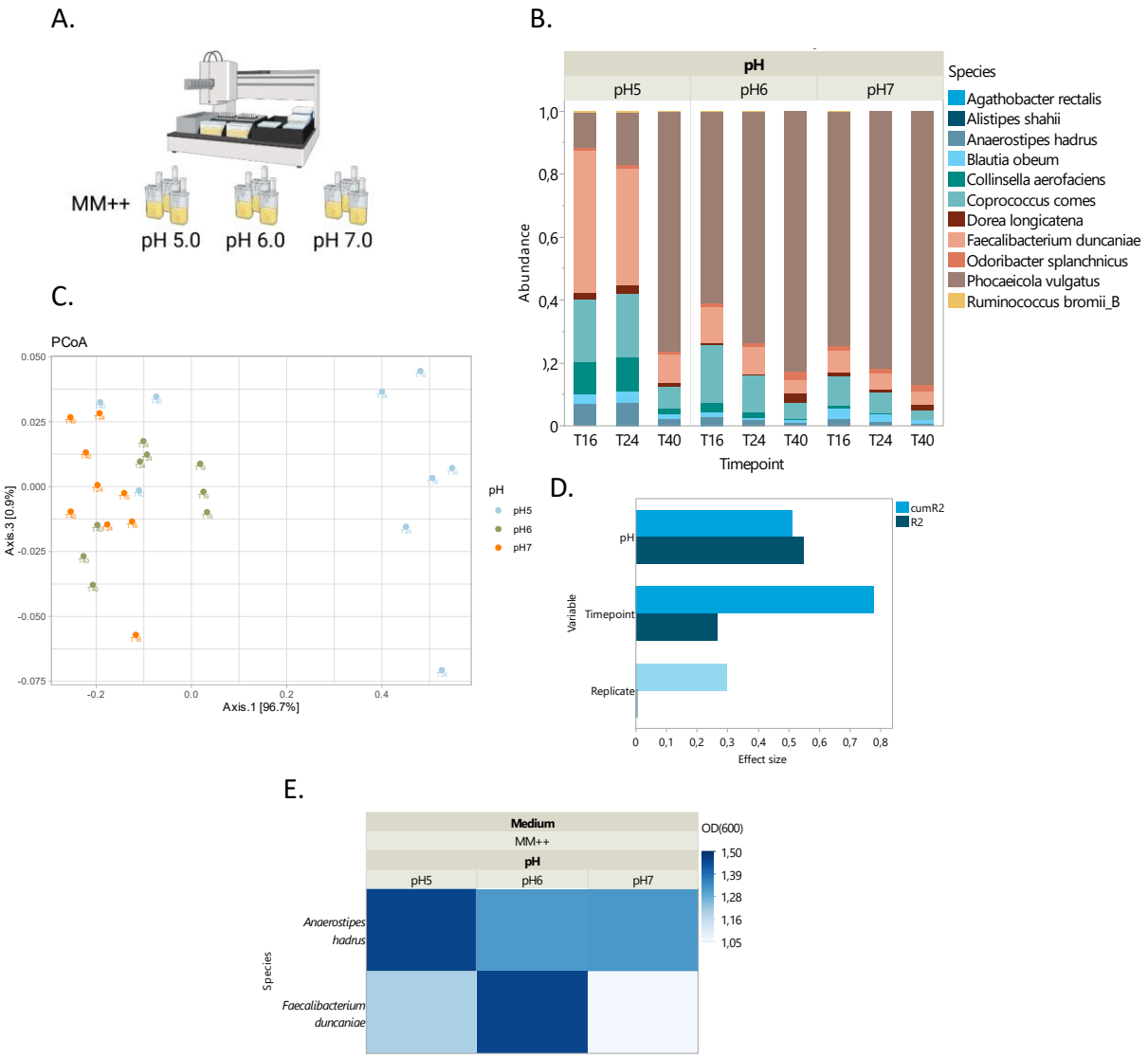


Figure 9. A. Experimental design: the M-HAEFC community (M1) was grown for 40 hours (MM++, pH 5.0, 6.0 or 7.0) in triplicates. Samples were taken for community and metabolic profiling at 16, 24 and 40 hours, 10% of the medium was replaced at 16 and 32 hours. The community was cultured using the Ambr® 15f platform. Image made with Biorender. B. Community composition at species level over time per pH (average composition of the triplicates). C. Species-level community variation of the fermentations in triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different pH (colours). D. Variables contributing most to compositional variation in the fermentations (dbRDA, species level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes) not significant data in light colours $p > 0.05$. E. Growth of *Anaerostipes hadrus* and *Faecalibacterium duncaniae* (OD_{600}) in different pH after 20 hours in MM++ (average of triplicates).

The pH-specific changes in community composition also affected metabolic production profiles. At pH 5.0, significantly higher levels of lactate and lower levels of acetate, butyrate, and succinate were produced compared to pH 6.0 and 7.0 (Kruskal-Wallis $p < 0.001$ – post hoc Dunn test $p < 0.05$). Propionate was measured in lower levels compared to pH 7.0 (Kruskal-Wallis $p < 0.001$ – post hoc Dunn test $p < 0.001$, Figure 8B). Overall, both formate and lactate decreased over time (Kruskal-Wallis $p < 0.02$, post hoc Dunn test $p < 0.03$) between 16 and 40 hours.

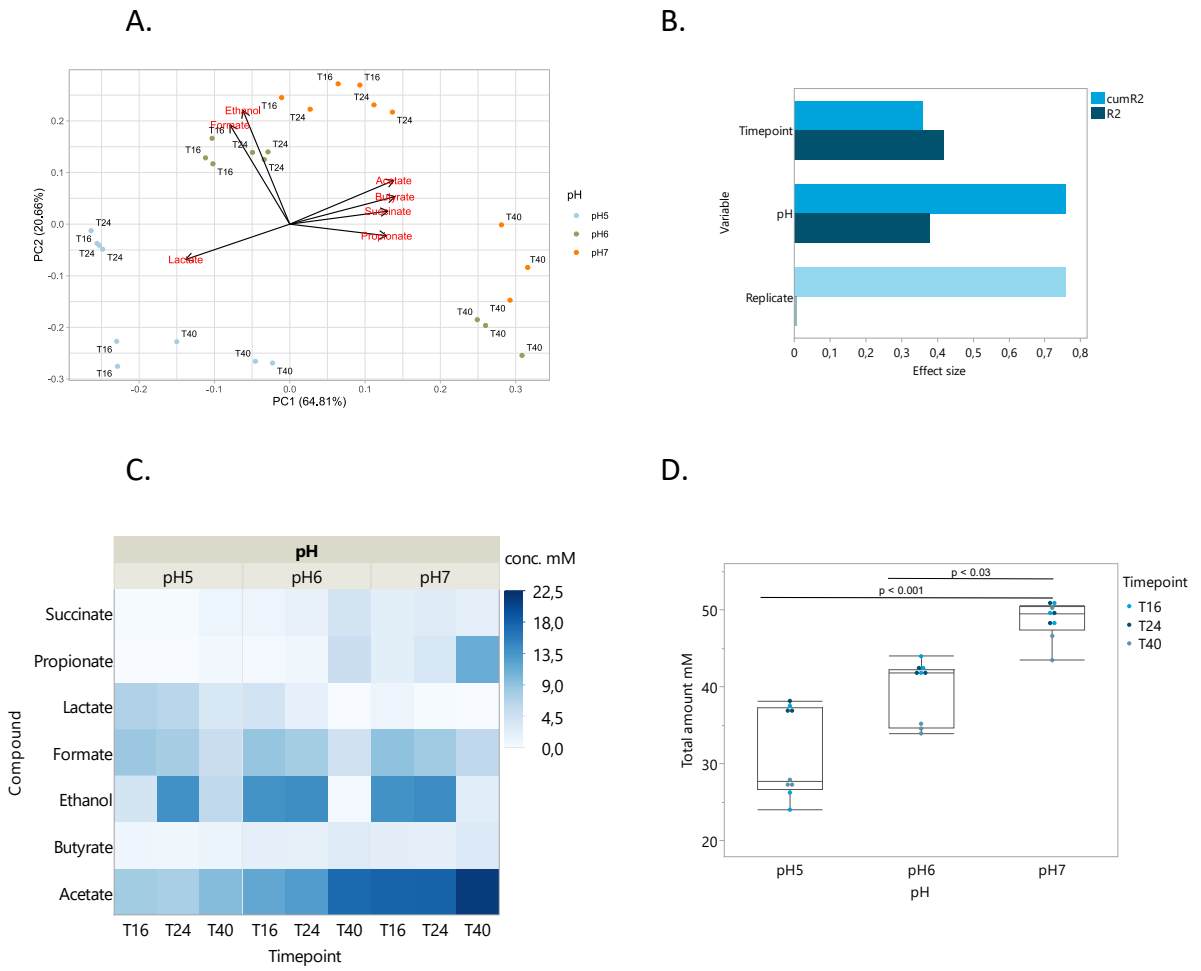


Figure 10. A. Variables contributing most to metabolic profile variation in the fermentations (dbRDA, Euclidean distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes) not significant data in light colours $p > 0.05$.B. Metabolite composition variation of the fermentations in triplicates, represented by principal coordinates analysis (Euclidean distances, PCA). Shown are samples from the different pH (colours). C. Average metabolic profiles for each community over time (in triplicates) D. Concentration of metabolites produced (mM) at all timepoints (coloured) per pH (Kruskal-Wallis $p < 0.001$, post hoc Dunn test $p = 0.02$ and $p < 0.001$).

The total amount of metabolites was significantly lower at pH 5.0 compared to pH 7.0 and 6.0, and higher at pH 7.0 compared to pH 6.0 (Kruskal-Wallis $p < 0.001$, post hoc Dunn test $p < 0.01$, Figure 8C). Therefore, time had the larger effect on functional profiles, with clear differences between pH 5.0 and other pH values tested (Figure 8D).
Since the starting pH was shown to affect both community composition and metabolic production, the effect of continuous pH control or the lack thereof was also examined (Figure 11 A). Here, the Ambr® 15f robot platform was used to maintain a pH range between pH 6.0 and 6.3 whereas fermentations without pH control were performed in Hungate tubes for 24 hours. In both setups, 10% feeding was done at 16 hours. The fermentation type (Hungate vs Ambr® 15f) alone significantly explained the difference in community composition (dbRDA effect size = 74%, Figure 11 B and C), replicates and timepoint were not significant variables.

Several species, including *Ag.rectalis, Alistipes shahii, F. duncaniae, R. bromii* and *An.hadrus*, were found to be more abundant in fermentations without pH control (Kruskal- Wallis post hoc Dunn test $p < 0.05$). Conversely, *D. longicatena* and *Cop. comes* were less abundant in communities with no pH control, as well as *Pho. vulgatus* and *O. splachnicus* (Figure 11 D, Kruskal- Wallis post hoc Dunn test $p < 0.05$). Only *Col. aerofaciens* and *B. obeum* were not significantly affected by the pH control in the tested conditions.
Overall, in these conditions (at pH 6.0), more species were detected above detection level in the community with no pH control.

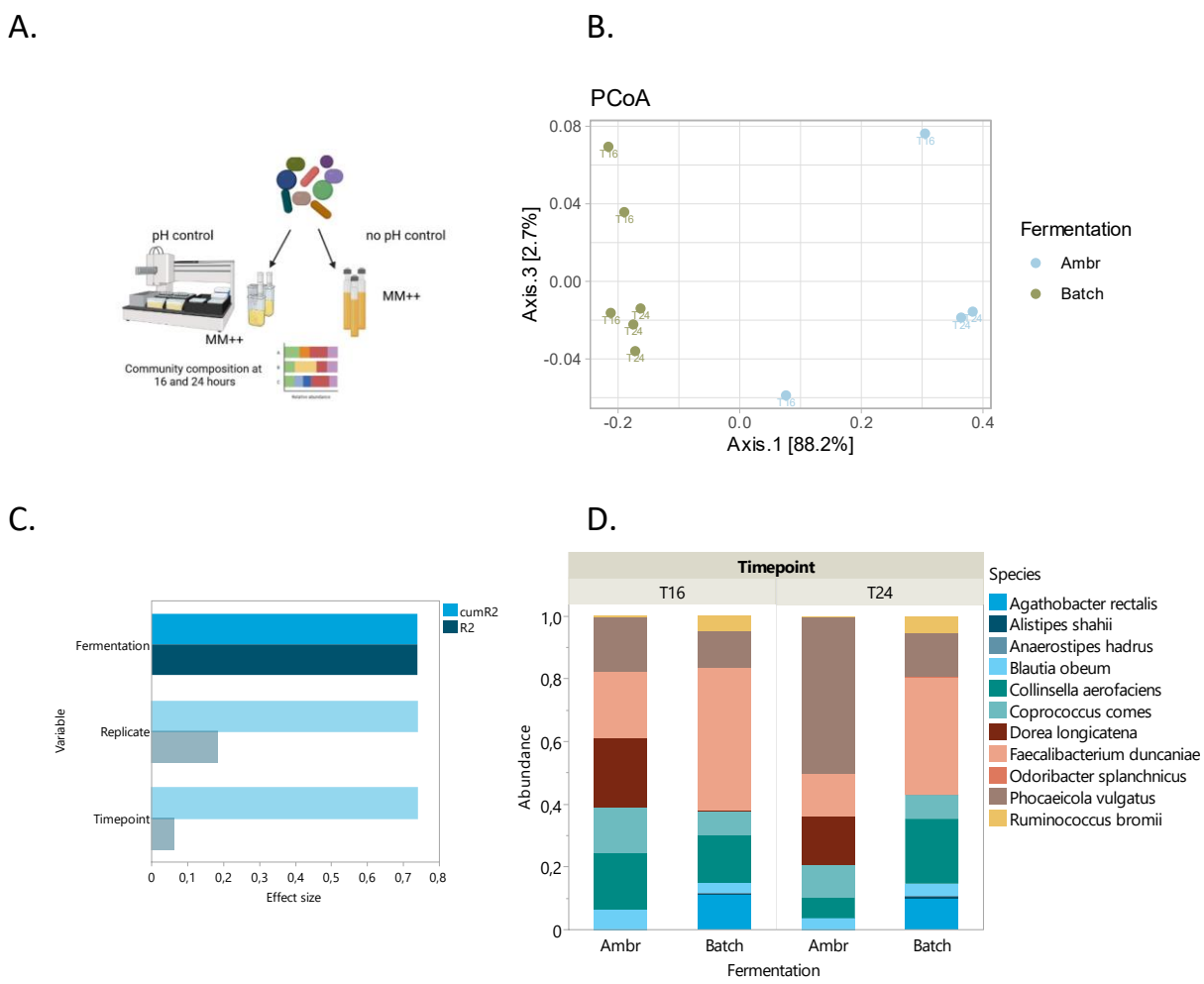


Figure 11. A. Experimental design: the M-HAEFC community (M1) was grown for 24 hours (MM++, pH 6.0) in triplicates with or without pH control. Samples were taken for community at 16 and 24 hours, 10% of the medium was replaced at 16 hours. Image made with Biorender. B. Species-level community variation of the fermentations in duplicates and triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different fermentation systems (colours). C. Variables contributing most to compositional variation in the fermentations (dbRDA, species level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes) not significant data in light colours $p > 0.05$. D. Community composition at species level at 16 and 24 hours in a continuous pH control system (Ambr®15f) and no pH control (fermentation in Hungate tubes).

Discussion

The human gut microbiota is known to play a crucial role in maintaining human health. To take a step towards designing safe and standardised new biotherapeutics based on defined communities, two minimal health-associated functional cores (M-HAFC) were selected based Bact1 and Prev enterotype core species and their functional capabilities.

The selected species were then studied at different levels of complexity, starting with monocultures to determine their substrate utilisation and metabolic output. This information was used to create a medium composition suitable for community assembly as it was able to sustain the growth of all species and the resulting communities enabled co-existence of all members of the community. Therefore, previous functional knowledge about the species and validation in vitro provided enough information to design the medium successfully.

The next level of complexity involved performing co-cultures to identify in vitro cooperation and competition behaviours. Two species, *Collinsella aerofaciens* and *Dorea longicatena*, were highly cooperative in silico, while in vitro results were less significant. Additionally, a lot of competition was observed in the co-cultures which did not result in exclusion of taxa during the community assembly. As previously described in the literature, pairwise interactions do not always reflect complex community interactions in vitro[173,174] and in vivo[175]. These results provide valuable knowledge into bacterial interactions but are not necessary in future designs towards building complex communities.

Before assembling the full community, four-member communities were tested, to ensure co-existence within different trophic levels. This experiment showed that functional complementarity (i.e., using members of the community with different functional niche as defined in Chapter 3 with the different functional clusters, Chapter 3 Figure 2) enabled communities to co-exist. The resulting 10 to 11 species community was resilient to perturbations, with a change in species showing that similar functional species could occupy the same niche and therefore not drastically change community composition. However, to better capture the community dynamics overtime, absolute counts should be added in the future. In this study, we observed an increase in relative abundance of *Pho. vulgatus*

compared to *F. duncaniae* in the Bact1 M-HAEFC, this effect was not observed in the Prev M-HAEFC. Absolute abundances rather than relative would add insights on the two communities.

Additionally, to further understand species interactions, future studies should include other omics analysis such as metabolomics and transcriptomics. The results of this study suggest that functionally similar species tend to occupy the same niche in a community, without disrupting its overall composition as using closely related species did not affect community composition in both the Bact1 and Prev-like communities. This finding is of great importance when selecting species for designing bacterial therapeutics, as each application may require a different set of species. For example, in IBD dysbiosis is characterised by a lower abundance of *Lachnospiraceae* and *Ruminococcaceae*[176] while in depression it has been reported that *Coprococus* and *Dialister*[18] were found in lower abundance. In conclusion, our results suggest that the pursuit of a functional community may be more relevant for next-generation therapeutics, rather than a taxonomic one[10,151,161].

Then, the study suggests that the Bact-1 like community was resilient in the tested conditions to antibiotics and opportunistic bacteria, here *E. coli*. Only time was a significant variable, while replicates and perturbation did not explain the variability seen in the community composition and function. Nevertheless, it is worth noting that the replicates did show a significant univariate effect, due to one of the replicates in the ampicillin group with a higher proportion of *F. duncaniae* before the perturbation. Therefore, this experiment could be repeated to further validate these results with additional replicates. Testing other strains of *E. coli* could also confirm the resilience of the community as the effect could be strain dependent. Indeed, it is necessary to test the resilience of communities for future biotherapeutics in a dysbiotic community, while this perturbation was a first step toward this goal, the community should be tested in vitro and in vivo in a dysbiotic environment to evaluate if it could potentially engraft or at least help in restoring a more balanced community.

Finally, these experiments suggest that the experimental conditions (e.g., pH) have an impact on the final community composition and should be tailored based on the desired outcome of the community, such as the production of specific metabolites or the growth

of particular taxa. The impact of pH on individual species and communities was already described in other studies[171,177]. However, quantitative results should be investigated rather than relative to better understand the pH differences on the communities. Unfortunately, it was not possible with our sequencing pipeline in this study. As the resulting community composition depends on these conditions, future studies should not overlook this aspect and find the adequate culture conditions for the desired purpose. This aspect will also need to be addressed in in vivo settings where conditions, such as pH, fluctuate in the gut environment.

Conclusion

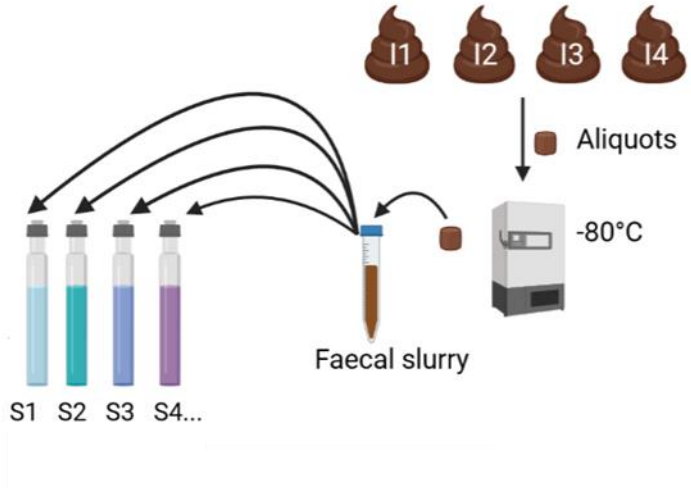
In conclusion, this study successfully assembled two defined bacterial communities that characterise the Bact1 and Prev enterotypes. Substrate utilisation and metabolic output analysis of monocultures, as well as in silico predictions, provided useful information for creating a successful medium composition. Finally, we showed that functional niches were more critical than specific taxa in designing defined communities, which has implications for the design of bacterial therapeutics where different sets of species may be required.

Chapter V

Substrate-driven propagation of bacterial communities enriched from human gut microbiota

This chapter is being prepared to submit for publication:

Anaïs Biclot; Gwen Falony; GinoVrancken; Raul Y. Tito; Sara Vieira-Silva; Geert R.B. Huys; […] Jeroen Raes. Substrate-driven propagation of bacterial communities enriched from human gut microbiota


I1
I2
I3
I4
Aliquots
-80°C
Faecal slurry
S1
S2
S3
S4...

Abstract

Despite major advances in understanding drivers of composition and functional potential of the gut microbiota, factors influencing community-level responses to nutritional inputs remain understudied. In this study, we assessed founder versus nutrient effects using series of batch fermentations (n=48; n=360) to longitudinally assess the community dynamics (16S) and metabolic activity (metabolomics) (5 time points, yielding, 360 samples) in stool inocula from healthy adult subjects in the presence of a range of substrates, including mono- and polysaccharides, short-chain fatty acids produced in the gut environment and host-derived glycans. We find that donor, not nutritional intervention is the main determinant of overall post-intervention community composition. At the same time, a number of bacterial genera were consistently enriched across different inocula in the presence of a specific substrate such as *Flavonifractor* in erythritol. When comparing different substrates within in an individual stool sample, substrate was found to be the primary driver of community variation, while metabolomic outputs were influenced by substrate, pH and fermentation duration. Substrate-driven functional and phylogenetic shifts are correlated, with complex nutrients having a greater effect on composition and function compared to intermediate metabolites or simple molecules. At the same time, certain substrates show greater compositional effects than metabolic, or vice versa. Finally, the time and range of the effect of repeated batch cultivation showed that polysaccharides led to more diverse communities, while intermediate metabolites and simple molecules led to specialised communities compared to standard medium.

In conclusion, enriching complex gut communities in different nutritional conditions helps guide targeted nutritional intervention in the human gut and may reveal new isolation strategies of taxa with poorly documented substrate specificities.

Introduction

The human gut microbiota plays a critical role in maintaining the health and well-being of the host by influencing various physiological processes, including digestion, metabolism, and immune function. The study of gut microbiota interactions and their potential therapeutic benefits has gained significant interest in recent years to restore an imbalanced community, referred to as dysbiosis. To comprehend the aetiology of diseases and restore a healthy gut community, it is necessary to understand bacterial interactions within complex communities. These interactions can have a substantial impact on gut health and investigating them in controlled laboratory conditions can provide mechanistic insights. Bottom-up approaches in vitro can facilitate the study of a defined and carefully designed community. However, they are more difficult to achieve because the strains need to co-exist, and the medium needs to be carefully designed. Top-down approaches, on the other hand, are substrate-driven faecal enrichments that lead to self-assembled in vitro communities.

In vitro enrichment cultures provide a unique opportunity to investigate the interactions between different bacterial species and their environment, and therefore could lead to identification of novel bacterial therapeutics that can modulate the gut microbiota. In addition, various environmental factors, such as nutrient availability or pH, can be investigated on the growth and behaviour of the gut microbiota. Some studies have used this approach to investigate the impact of specific substrates, such as pectin[178], starch[179], or multiple fibres[180–182] found in the diet. These studies enable a better understanding of the substrate degradation capacity of community members and potential cross-feeding interactions, and coupled with metabolomics, unravel the community functional potential, mainly the production of short-chain fatty acids (SCFAs), which are beneficial for the host[183]. However, no studies to date have investigated the impact of multiple types of substrates on samples from different individuals (e.g., dietary fibres as well as SCFAs and food additives).

In this study, we used repeated batch cultivation, a widely used biotechnology technique also known as 'backslopping' for producing compounds such as biofuels or enriching particular microbial communities, such as during the sourdough process. The aim of the

study was to enrich for specialised communities and assess the effect of different substrates on resulting community compositions and metabolic profiles. We used different faecal inocula in several selected media, including dietary fibres (e.g., starch, pectin), host-derived glycans (mucin), and metabolites produced by microbes (e.g., acetate or lactate), typically used for cross-feeding interactions. We also tested other dietary molecules used as food additives, such as erythritol, to investigate their impact on bacterial communities.

Methods

Sample collection, selection and processing

Four stool samples (I1, I2, I3 and I4) were used in this study from healthy individuals and very high cell counts indicating they were non-dysbiotic[15,16]. All faecal samples were collected with collection kits[9,184] and stored at -20°C in the participants home, transported on dry ice and stored at -80°C in the form of pellets in the lab until processing. The cell density was measured by flow cytometry and the DNA was extracted as previously described[9] using the MagAttract PowerMicrobiome DNA/RNA kit (Qiagen, Germany).

Media preparation

For this study, twenty-four different media were prepared (Table 1). They include several polysaccharides, host-derived glycans, monosaccharides, short chain fatty acids found in the gut environment. The nutrient poor Luria-Bertani (LB) medium was used as a base medium and was diluted ½ to reduce the bloom of *Escherichia coli* observed during preliminary tests (final concentration of 10 $g.L^{-1}$). Next, 10 $g.L^{-1}$ stock solutions (filter sterilised, 0.2 μm) were prepared for the individual substrates and added to LB to a final concentration of 1 $g.L^{-1}$. Non-filtrable substrates (mucin, pectin and starch) were directly added to LB in a concentration of 1 $g.L^{-1}$ before autoclaving with 1.25 g/L of sodium bicarbonate (SIGMA-ALDRICH, France) was added to buffer the medium. All media were adjusted to pH 6.0.

Table 1: The 24 different substrates used in the media prepared with LB and each of the following carbon sources.

Substrate type	Medium	Details	Product reference
Baseline medium (LB)	LB	Base medium, contains peptone, NaCl, yeast extract	Luria Bertani broth Sigma-Aldrich
(poly)alcohol	Erythritol	Food additive	Sigma-Aldrich
(poly)alcohol	Ethanol	Microbial metabolite	Ethanol absolute, VWR
(poly)alcohol	Glycerol	Involved in bacterial phospholipid biosynthesis	Sigma-Aldrich
(poly)alcohol	Mannitol	Food additive	D-mannitol, Sigma-Aldrich
Disaccharide	Cellobiose	Dietary-derived molecule: conversion of cellulose into cellobiose	D-(+)-Cellobiose, Sigma-Aldrich
Disaccharide	Maltose	Dietary-derived molecule	D-(+)-Maltose monohydrate, Sigma-Aldrich
Disaccharide	Trehalose	Dietary-derived molecule also used as food additive for frozen products	Sigma-Aldrich
Host-derived glycan	Mucin	Most abundant macro-molecule in mucin in the GI tract, source of nutrients for some bacteria	mucin from porcine stomach Type II Sigma-Aldrich
Microbial metabolite	Acetate	Intermediate molecule used in bacterial metabolism	sodium acetate Sigma-Aldrich
Microbial metabolite	Butyrate	Molecule produced by bacterial metabolism, important for colonocytes	sodium butyrate Sigma-Aldrich
Microbial metabolite	Formate	Intermediate molecule used in bacterial metabolism	Sodium formate Sigma-Aldrich
Microbial metabolite	Hemin_Acetate	Acetate and hemin were added in the medium. Source of iron and intermediate molecule used in bacterial metabolism	50 mg of hemin (SIGMA-ALDRICH, China) was dissolved in 50 mL NaOH (Merck, Germany) 1M, sodium acetate Sigma-Aldrich
Microbial metabolite	Lactate	Intermediate molecule used in bacterial metabolism	sodium DL-lactate 60% (Alfa Aesar, Germany)
Microbial metabolite	Lactate_Acetate	Lactate and acetate were added in the medium. Intermediate molecule used in bacterial metabolism	sodium DL-lactate solution 60% w/w Sigma-Aldrich), sodium acetate Sigma-Aldrich
Microbial metabolite	Propionate	Molecule produced by bacterial metabolism, important for colonocytes	Sodium propionate, Sigma-Aldrich
Microbial metabolite	Succinate	Intermediate molecule used in bacterial metabolism	sodium succinate dibasic hexahydrate Sigma-Aldrich
Monosaccharide	Glucose	Energy source	D-(+)-Glucose, Sigma-Aldrich
Monosaccharide	Xylose	Dietary-derived molecule: conversion of xylan into xylose	D-(+)- xylose Sigma-Aldrich
Polysaccharide	Cellulose	Dietary molecule	Avicel® PH-101, Sigma-Aldrich
Polysaccharide	Citrus pectin (CP57)	Dietary molecule, CP57 57% degree of methyl esterification	LFT-SMART, KU Leuven, Belgium
Polysaccharide	Starch	Dietary molecule	starch from potato (soluble), Sigma-Aldrich, Germany
Polysaccharide	Xylan	Dietary molecule	≥95 % xylooligosaccharides, from corncob, Carl Roth
Nitrogen source	Urea	Main nitrogen-containing substance in urine	Urea solution, ~8M, Sigma-Aldrich

Inoculation and repeated batch cultivation

A frozen faecal pellet was weighted, dissolved in PBS (incl. vortexing) to a final concentration of 10^9 cells.mL^{-1}. The solution was filtered with a 0.5 µm filter to remove debris. The inoculate was added in 1/100 concentration to the prepared medium previously reduced overnight in the anaerobic chamber in Hungate tubes. All preparations for the inoculate and repeated batch cultivation were carried out in an anaerobic chamber at 37°C under anaerobic conditions (10% H_2, 10% CO_2, 80% N_2) in a Don Whitley A35 Anaerobic Workstation with HEPA filter (Don Whitley Scientific, Shipley, UK). After the inoculation, the tubes were closed with a cap and mixed to homogenise and incubated for 48 hours (timepoint 1). After 48 hours, 1 mL of the fermentation liquid was transferred to a new tube containing 9 mL fresh medium (preincubated overnight) for another 48 hours (backsloppping; timepoint 2, Figure 1). At timepoint 2, 1 mL of the fermentation was taken for DNA extraction and the pH was measured. In a second experiment only one sample was used (I1); the repeated batch cultivation was performed four times (up to timepoint 5, Figure 1). For this experiment, at all timepoints, 1 mL of the fermentation was taken for DNA extraction and HPLC analysis and pH was measured.

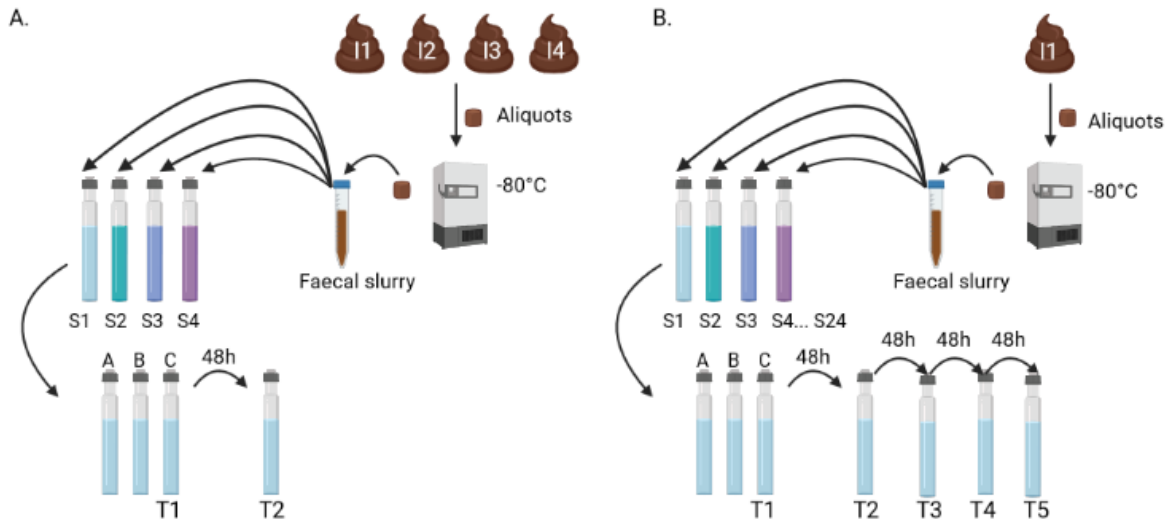


Figure 1. Experimental procedure: the faecal samples were aliquoted into pellets and kept at -80°C until processing. The aliquots were then diluted into PBS to have a final concentration of 10^9 cells.mL^{-1} in the faecal slurry to be inoculated in each medium after a 1/100 dilution. Every 48h, 10% of the solution was transferred into fresh medium. A. For the four inocula, 4 media were tested and with one step of repeated batch cultivation (to T2) and B. for the follow-up experiment 24 substrates were tested with only I1 and four steps of repeated batch cultivation were performed (to T5). Image made with Biorender.

Microbial community analysis

Fermentation samples were centrifuged, and supernatant was removed prior to storage at -80 °C. Frozen faecal aliquots (150200 mg) and fermentations were subjected to DNA extraction using the PowerMicrobiome RNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, USA) according to the manufacturer's instructions as described previously[9]. The V4 hypervariable region of the 16S rRNA gene was amplified using forward primer 515F (5'-GTGYCAGCMGCCGCGGTAA 3') and the reverse primer 806R (5' GGACTACNVGGGTWTCTAAT-3'), modified with adapters and barcodes. Sequencing was then performed using the Illumina MiSeq platform (MiSeq Reagent Kit v2, 500 cycles, 20% PhiX) according to the manufacturer's specifications to generate paired-end reads of 250 bases in length in each direction. After demultiplexing with sdm as part of the LotuS[132] pipeline without allowing for mismatches, fastq sequences were pre-processed using DADA2 pipeline v1.14.123. The taxonomy was assigned using GTDB release 202[135,166]. For relative microbiome analyses, each sample depth was downsized to 10,000 reads.

Metabolic readouts

Frozen supernatant was thawed from −80°C (30 minutes, room temperature). Concentrations of glucose, ethanol, succinic acid, lactic acid, formic acid, acetic acid, propionic acid, and butyric acid in the supernatant were measured on an Agilent 1200 HPLC (Diegem, Belgium) system equipped with an Aminex HPX-87 H column (Bio-rad, Temse, Belgium). The column was kept at 40°C and eluted with 5 mM H_2SO_4 at a rate of 0.60 $mL.min^{-1}$. Organic- and fatty acids, glucose and ethanol were detected with refractive index changes (Agilent RID, at 40°C).

Statistical analysis

All statistical analyses and graphical representations were performed in R using the packages phyloseq[137] vegan[138] and ggplot2[140]. Observed richness was calculated with the R package phyloseq. Microbiome variation between individuals was visualised by PCoA using Bray-Curtis distances, only samples with more than 10,000 reads and amplicon sequence variants (ASV) with a relative abundance higher than 0.001 were included in the 16S data analysis. The contribution of metadata variables to the community variation was determined by dbRDA on genus-level Aitchison distance (Bray-Curtis distances between samples after

rarefication) with the capscale function in the vegan R package[138]. Correction for multiple testing (Benjamin–Hochberg procedure, FDR) was applied and significance was defined at FDR < 0.1. The cumulative contribution of metadata variables was determined by forward model selection on dbRDA with the ordiR2step function in vegan[138], with variables that showed a significant contribution to community variation in the previous step. Alpha-diversity, number of observed genera (richness) were determined using the vegan R package[138].

Results

Interindividual variation is stronger than the effect of substrate

First four faecal samples from different healthy individuals (I1, I2, I3 and I4) were cultured in four different media containing LB and one specific additional substrate (i.e., erythritol, starch, mucin and trehalose, Figure 1A). These substrates represent different nutrient source types found in the gut environment (substrate type): food additives, polysaccharides, host-derived glycans and disaccharides. The four samples were similar in initial composition (i.e., stool samples) having a high proportion of *Faecalibacterium, Phocaeicola* and *Agathobacter,* and fall within the *Bacteroides* 1 enterotype (Figure 1 A.). Samples were inoculated at 10^7 cells/mL and subjected to a single backslopping with two 48h fermentations in each enriched medium.

The variation in community composition was significantly explained by the individual, medium, and pH after fermentation, accounting for 70% of the variation (Figure 1B). The replicates were not significantly different which showed that the communities behaved similarly each time. However, the individual alone explained approximately 50% of the variation, while medium only an additional 17% (distance-based redundancy analysis (dbRDA, Figure 1B.). This result could also be observed by examining the average Bray-Curtis (BC) distances between communities (each set of triplicates), where samples clustered more by individual than by medium (Figure 1C & D, confirming that the intervention could not overcome interindividual differences.

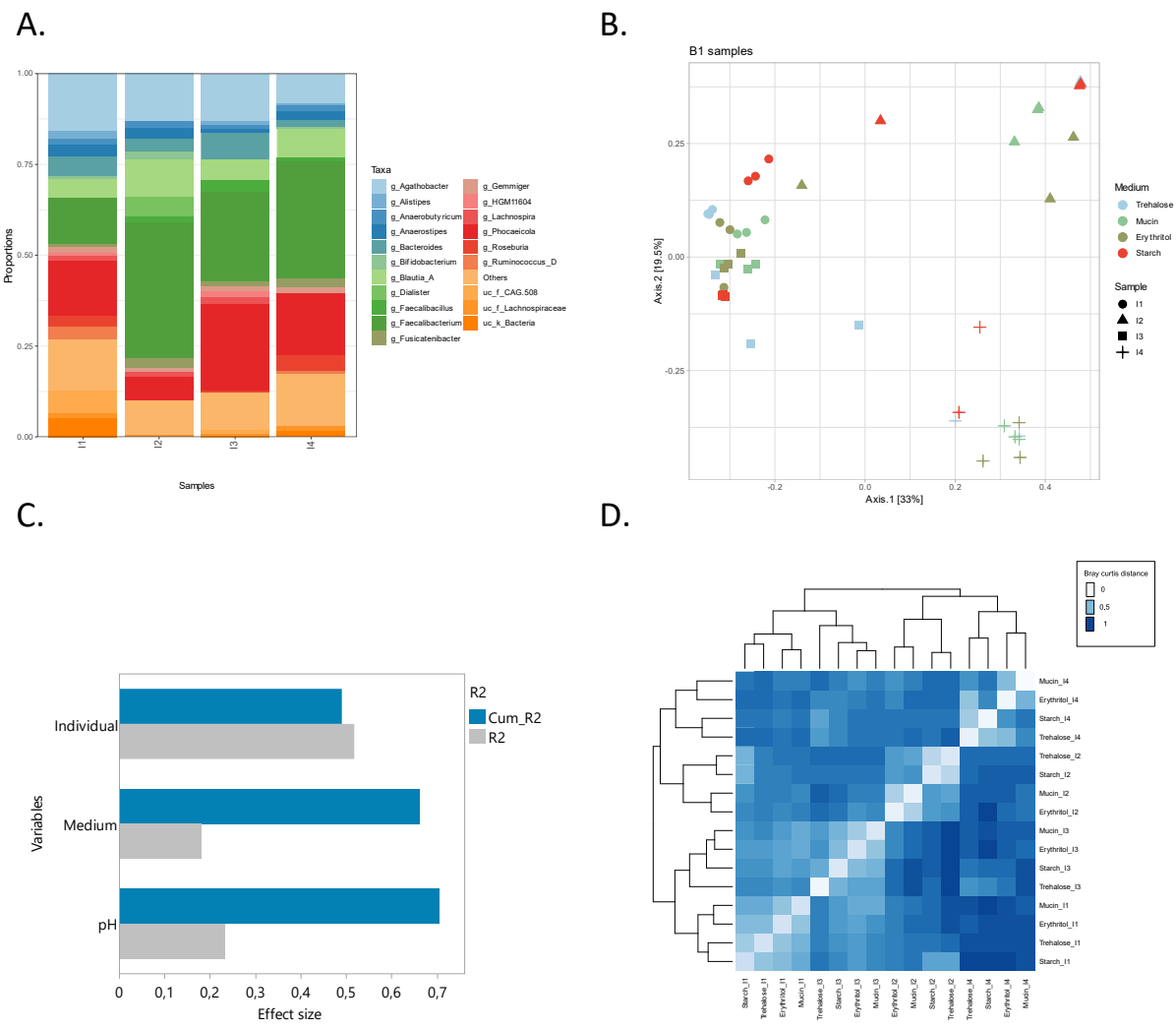


Figure 1. A. Relative abundances of the top 20 genera from the 4 selected faecal samples used for inoculation based on 16S rRNA gene community profiling B. Genus-level community variation of the fermentations in triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different samples (shapes) and media (colours). C. Significant variables contributing most to compositional variation in the fermentations (dbRDA, genus level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes) p <0.05.D. Bray-Curtis distances between all media from all individual samples clustered by closest distances.

By looking at specific taxa, specific substrate effects can be identified where some genera were enriched in the same medium in all individuals such as *Flavonifractor* in erythritol, *Parabacteroides* in mucin or *Bacteroides* in mucin and erythritol (Figure 2A). However, enrichments were mostly not consistent as only these three genera showed to be enriched in the same medium in all individuals. For example, the genus *Akkermansia* was found enriched in the mucin substrate of two out of four individuals (I1 and I3) (Figure 2 A and B), although I4 also had *Akkermansia* present in the baseline sample. One major difference among these samples was the pH after fermentation. In all samples but I4, the pH was around 6.5, while for I4 it was around 5.5 (Figure 2 C). Previous studies have shown that in monocultures the optimal pH for *Akkermansia* is 6.5, and less growth is observed at lower pH[177,185]. Therefore, in I4 it is possible that other members of the community produced a higher amount of acids that lowered the pH which disadvantaged *Akkermansia* and led to it being outcompeted.

These results indicate that the backslopping technique can yield relevant information of nutrient use and its community-wide effects in faecal ecosystems, but that this signal is overclouded by individual signals. Therefore, we next focused on a broader and longer experiments focusing on samples from a single individual.

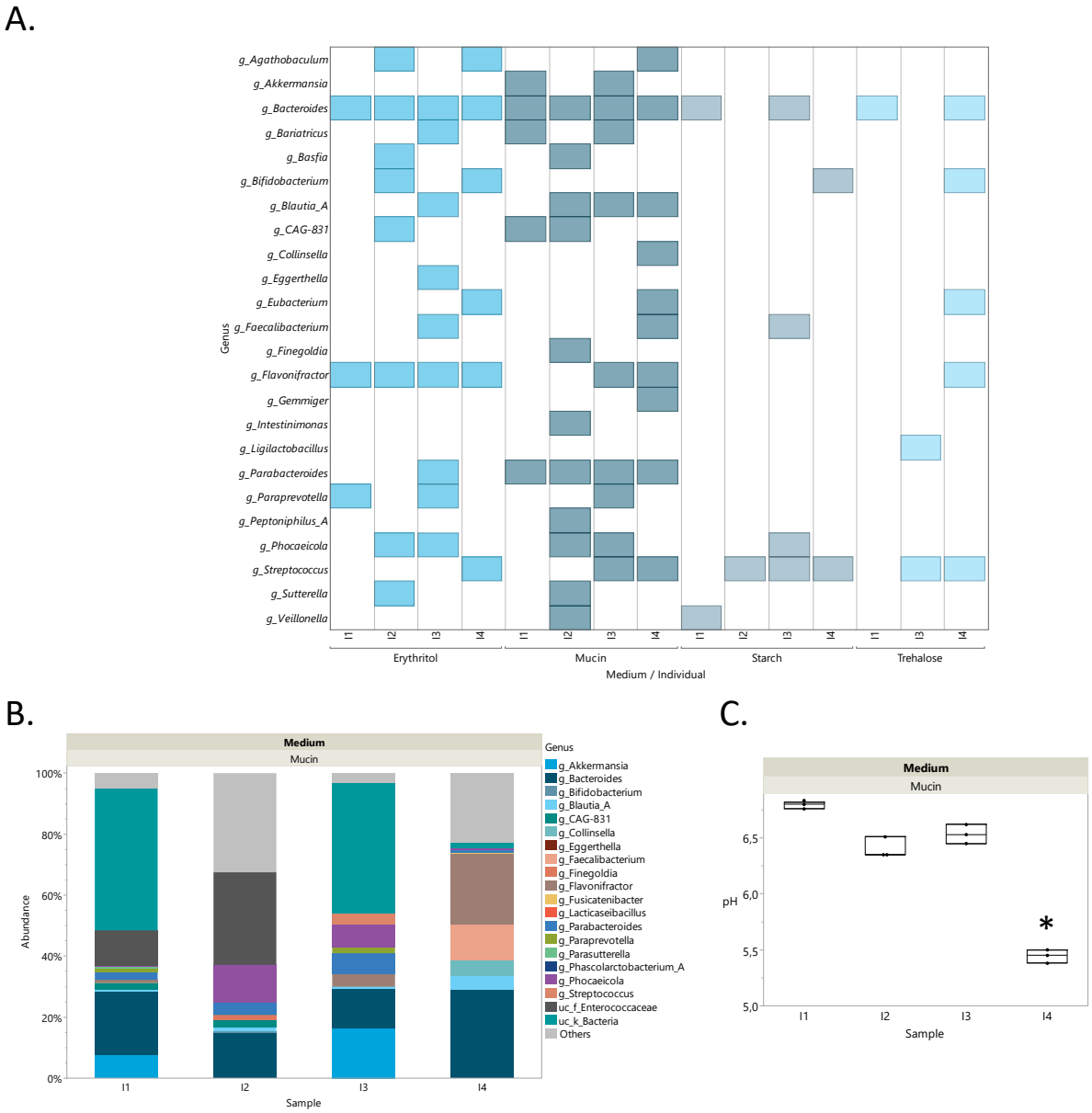


Figure 2. A. Presence/absence of each genus per individual and per medium (detection threshold >0.01%). B. Relative abundances of the top 20 genera from the 4 individuals fermented in mucin (average of the triplicate) derived from 16S rRNA gene community profiling. C. pH at end of each fermentation per individual in mucin (in triplicate).

Different media lead to different communities

Next, we subjected sample I1 to 24 substrates, or combination of substrates (Table 1, Figure 1B). The tested substrates were added to a base medium (LB) and comprised various substrate types including mono-, di-, or polysaccharides (e.g., glucose, maltose or starch), microbial metabolites (e.g., SCFAs), food additives (e.g., erythritol) and host-derived glycans (mucin). These substrates are derived from the diet or microbial metabolism in the gut (details in Table 1). This time the repeated batch cultivation step (10% of fermentation transferred into fresh medium) was executed five times to maximally drive the community to specialise into the specific substrate by adding a stronger bottleneck on the community. Each timepoint was subjected to sequencing and metabolomics analysis.

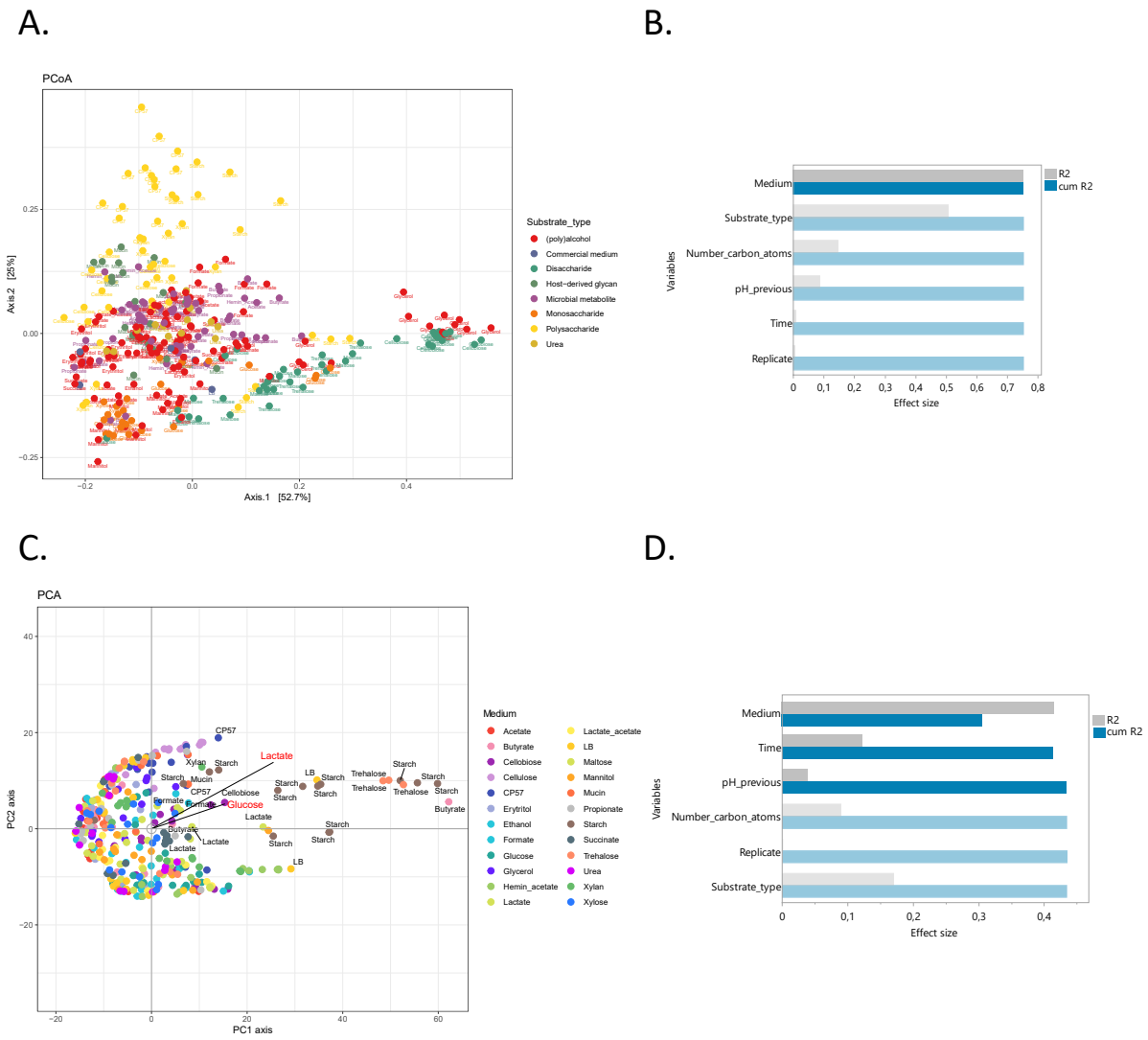


Figure 3. A. Genus-level community variation of the fermentations in triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different substrate types (colours). B. Variables contributing most to compositional variation in the fermentations (dbRDA, genus level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes), not significant variables are in light colours ($p > 0.05$). C. Metabolite composition variation of the fermentations in triplicates, represented by principal coordinates analysis (Euclidean distances PCA). Shown are samples from the different media (colours). D. Variables contributing most to metabolic composition variation in the fermentations (dbRDA, genus level Euclidean distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes), not significant variables are in light colours ($p > 0.05$).

We find that communities assembling in different growth media were different from each other with the substrate having the largest effect size on community variation (~75%) as the only significant variable (dbRDA $p < 0.05$, Figure 3 B). However, the broad category of substrate type alone (i.e., monosaccharide or polysaccharide) did not significantly explain the differences in communities (dbRDA $p > 0.05$, Figure 3 B).

In terms of metabolite production, the medium had the largest effect on the resulting metabolome, explaining 31% of the variation, followed by time and pH (pH_previous, the pH from the previous timepoint), which explained an additional 12% of the variation (dbRDA $p < 0.05$, Figure 3D). Again, the substrate type did not significantly explain metabolic variation. Lactate and glucose were explaining the difference seen in fermentation containing trehalose and starch, with high lactate and glucose concentrations ($p < 0.01$, Figure 3C, Supplementary Figure S5.1) resulting from both backslopping procedures. Glucose was only accumulated in these two communities and was probably only partially consumed which led to accumulation. Both media, trehalose and starch, also were enriched in lactate producers, *Bifidobacterium* and *Ruthenibacterium* in starch and *Lacticaseibacillus paracasei* in trehalose (Supplementary Figure 5S.2), likely explaining the accumulation of lactate in the medium.

Community specialization: enrichment compared to LB

The metabolic production profile distances (Euclidean (Eu) distances) and community profile distances (Bray-Curtis (BC) distances) were calculated between each medium and were found to be correlated (Spearman $\varrho = 0.289$, $p < 0.0001$, Figure 4A) indicating a clear relationship between function and composition. Most media were similar in both function and composition (low distances), but some such as starch and hemin_acetate had a high metabolic dissimilarity to all other media given the compositional similarity (Figure 4A). Metabolomics analysis showed that the starch intervention led to higher levels of acetate, formate, glucose, and lactate compared to all other media (Figure 4A). Moreover, this medium also caused a larger compositional shift as all comparison fell above the BC distance median. Lactic acid producers dominated in starch, mainly *Bifidobacterium* and *Ruthenibacterium,* both of which were mainly found in this medium (>80% of the total relative abundance of each was found in starch, Supplementary Figure S5.2). For hemin_acetate, one product, ethanol, was significantly higher compared to other media (t-test, $p<0.0001$, Figure 4C). However, no enriched taxa were found, which could indicate a change in metabolism rather than a change in composition within the community. Interestingly, hemin_acetate and LB showed the lowest BC distance (=0.09) but the second highest metabolic difference (Eu = 0.66).

In comparison to other media, cellobiose, pectin (CP57) and glycerol showed a large compositional shift (Figure 4A, Supplementary Figure 3 and 4A). The bloom of *Enterococcaceae* in cellobiose and glycerol, in contrast to *E. coli* in other media (Supplementary Figure 3B and 4), likely accounts for the high compositional distance observed. In the case of pectin (CP57) the significant compositional shift compared to other media is due to a high richness and taxa uniquely found in this medium (Supplementary Figure S5.2 and S5.5).

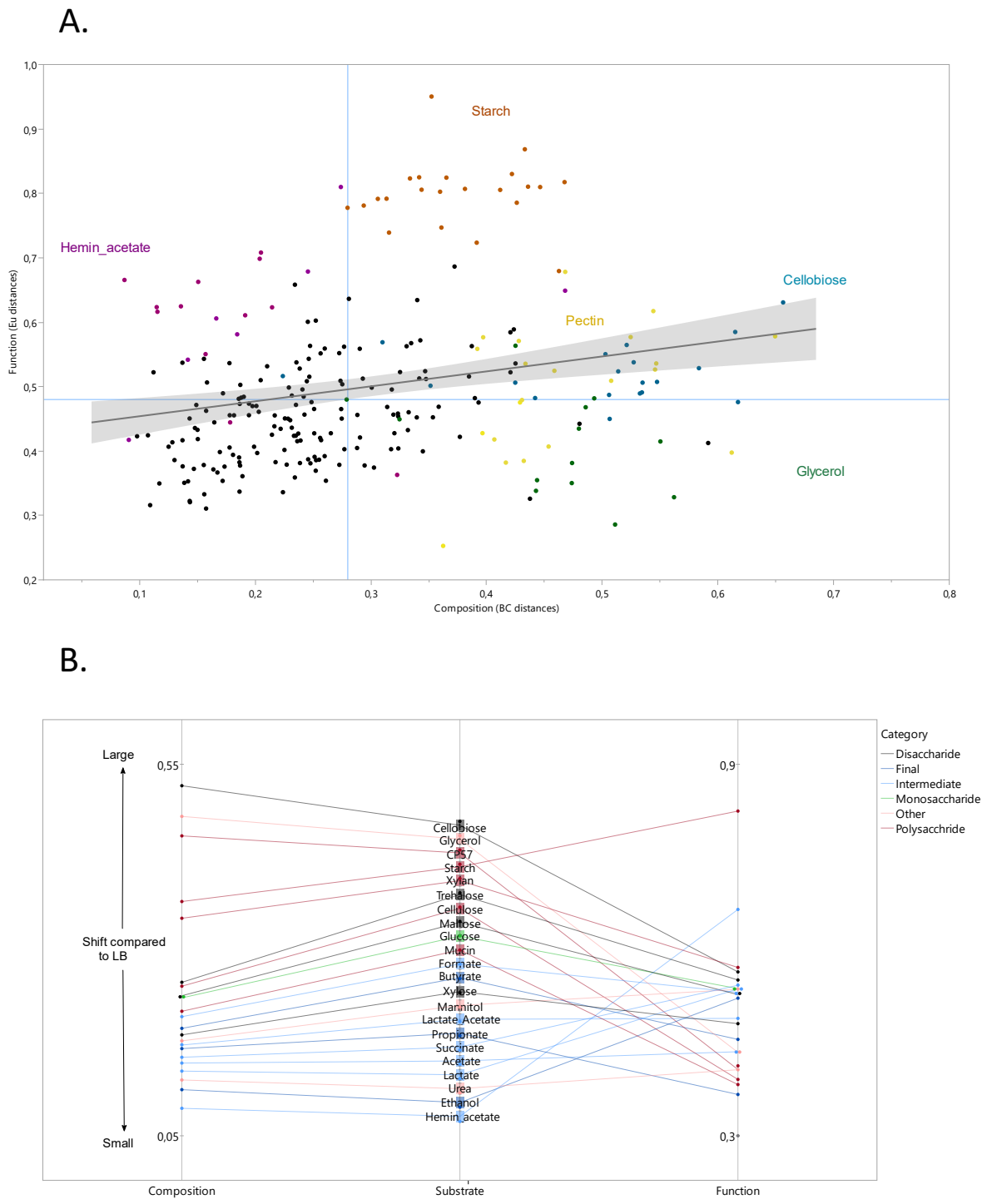


Figure 4. A. Metabolic production distances (Euclidean distance) and compositional distance (Bray-Curtis dissimilarity) averaged between each medium, the blue lines represent the median Eu and BC distances. B. Compositional distance (Bray-Curtis dissimilarity) of each medium to LB (Composition left), and metabolic production distances (Euclidean distance) of each medium to LB (Function, right) linked per medium.

Next, as all substrates were added to LB medium as the base medium, comparing the differences in community composition to the LB community enabled to better understand the impact of each substrate on the communities (significant taxa compared to LB only, Kruskal-Wallis with post hoc Dunn test $p < 0.001$; $p < 0.05$). Overall, polysaccharides (e.g., pectin) showed to be more distinct in terms of community compositions (Figure 4B), while microbial metabolites (e.g., acetate) were more similar to LB and therefore having a lower effect on shifting the community. The only exceptions were cellobiose and glycerol, but their BC distance is mostly driven by the bloom of *Enterococcus* as previously discussed. Therefore, there is a clear impact of substrate complexity on community and functional shifts. Moreover, out of the 23 substrates tested, only 16 (approximately 2/3) media had at least one taxon that was significantly increased in the substrate compared to LB (Figure 5A).

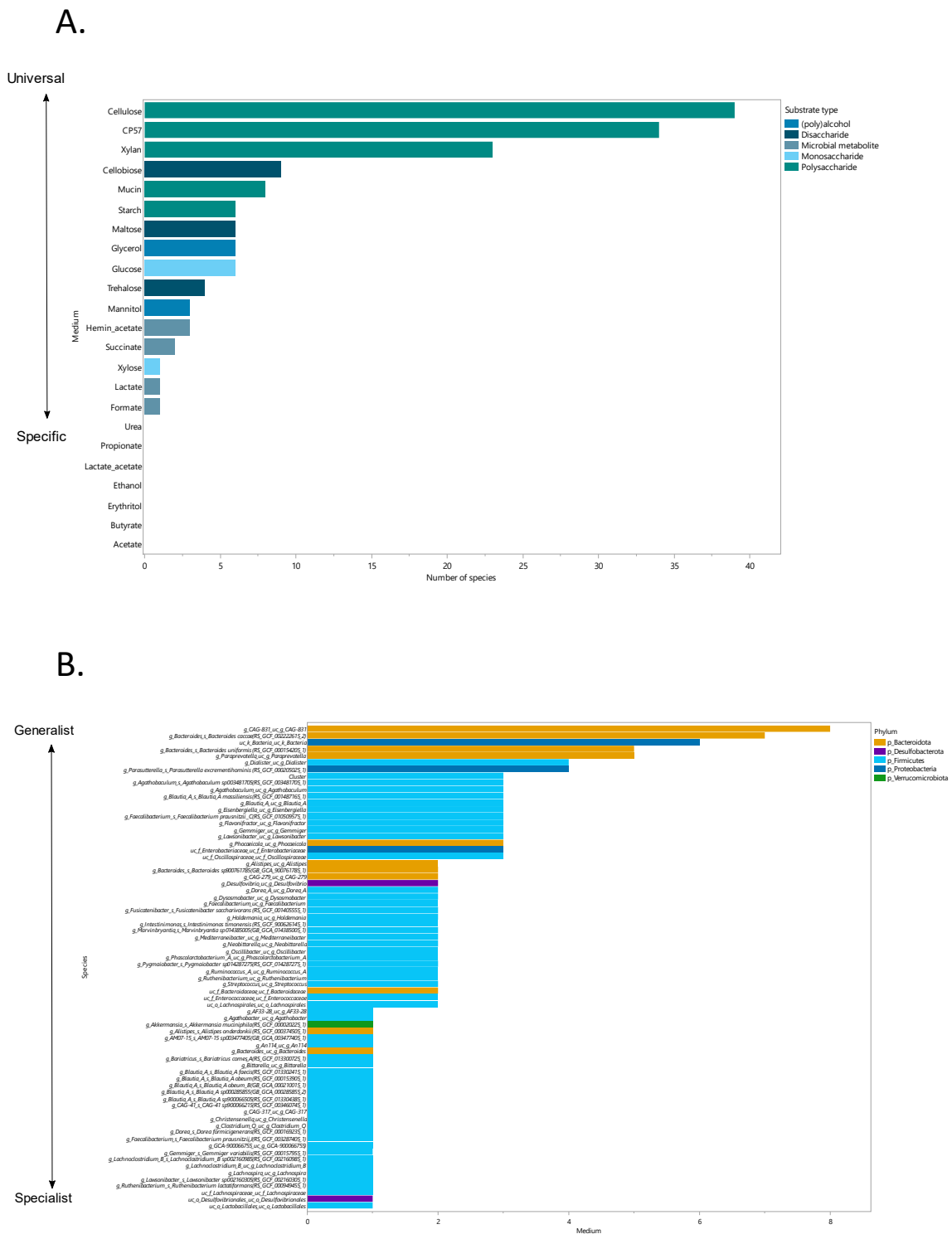


Figure 5. A. Number of significantly enriched species compared to LB per medium coloured by substrate type B. Number of media in which each species was significantly increased compared to LB.

After ranking each of the 16 media by the total number of significantly enriched taxa from LB, we found that cellulose, pectin (CP57) and xylan stimulated growth of the largest number of taxa (n=39, n=34, n=23 respectively). In contrast, other substrates such as formate, lactate and xylose only enriched a single species (Figure 5A). Therefore, the former substrate could be regarded as more universal media, leading to a more complex community. This could be due to the presence of multiple taxa capable of degrading the substrate or the formation of an interacting community with a few specialists that degrade the substrate and promote the growth of cross-feeding partners. Here again, we observed that polysaccharides led to more diverse communities compared to LB, while intermediate metabolites (e.g., lactate) or small molecules (glucose) were more similar to LB and therefore having a lower effect on shifting the community.

On the other hand, if we consider species that were significantly enriched compared to LB, we observe that some, such as CAG-831 (*Parabacteroides merdae*), were found in eight different media, whereas many others, such as *Lactobacillus* or *Desulfovibrio,* were found in only one medium (Figure 5B). This indicates that CAG-831 is more of a generalist that can degrade multiple substrates, whereas *Lactobacillus* or *Desulfovibrio* can only degrade one or find cross-feeding partners in limited conditions. Overall, more Firmicutes were found to be specialists (found in few media), while Bacteroidetes were found to be more generalists (found in many media) as previously reported[186].

This shift from LB was dependent on time for many substrates, with more taxa being enriched in the different substrates overtime. The enrichment overtime is seen in many substrates already from T02 with less increase in other timepoints except for pectin (CP57) or succinate. For some substrates such as cellobiose or mucin, the number of taxa enriched is stable from T01. Only one substrate (xylose), showed less species enriched after T01. Repeated batch cultivation provided an opportunity for the communities to specialise more using their substrates by diverging from the LB only community (Figure 6, details of the species in Supplementary Figure S5.7).

Conversely, a few species were found lower in abundance in all media compared to LB. These taxa were then able to grow well on LB only and were found in all other substrates but at a lower abundance: *uc_g_Bacteroides, g_Bacteroides_s_uniformis, uc_g_Paraprevotella,*

uc_g_CAG-831, g_Phocaeicola_uc_g_Phocaeicola, g_Bacteroides_s_caccae. These organisms are classified under the order Bacteroidales, which typically consists of more generalist species that can thrive with less competition in the medium. However, the relative abundance of these generalists may decrease in other media where the substrate favours specific taxa.

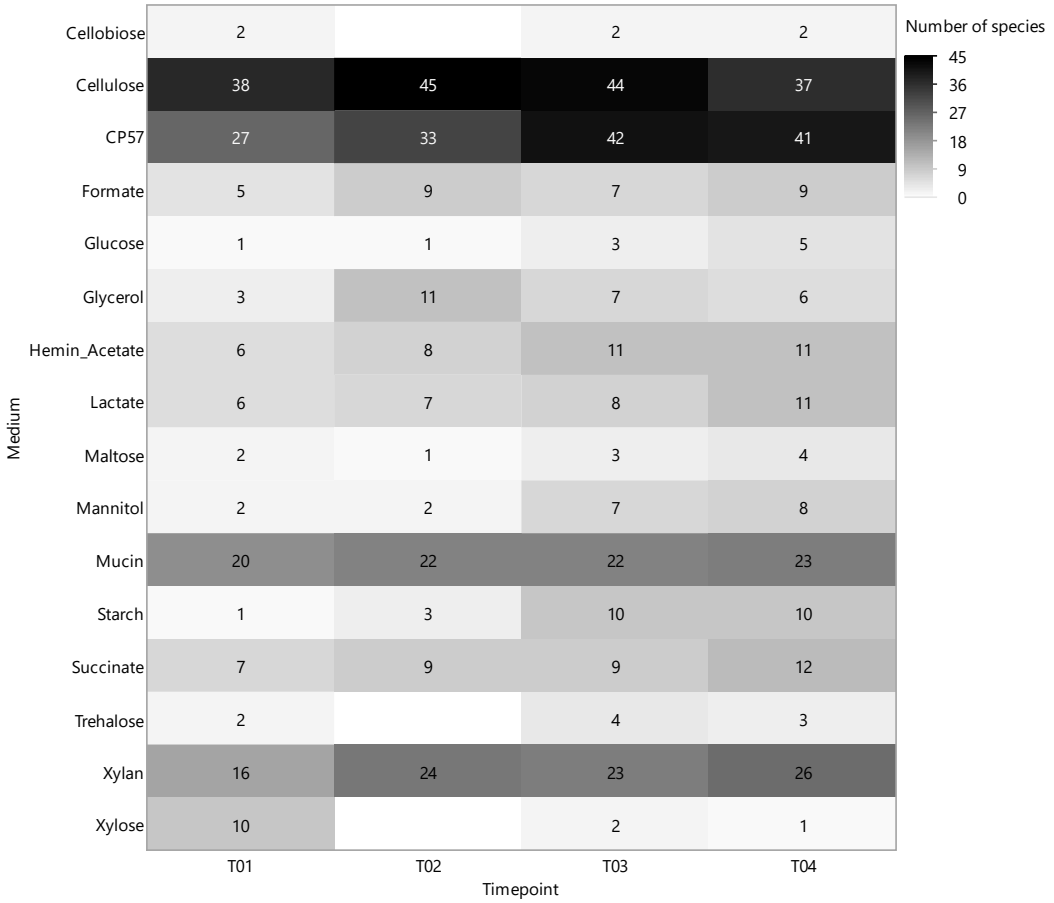


Figure 6. Heatmap of the difference in number of species significantly enriched in each medium overtime compared to LB.

Discussion

This study investigated the impact of different substrates added to LB as a basal medium on self-assembled faecal microbial communities and their functional profiles. The first experiment involved four faecal inocula and four substrates. The results showed that interindividual variation had a stronger influence on the microbial community than the substrates as previously reported[187,188]. Nevertheless, some taxa could be found to be similarly enriched in the same substrate from all the donors (e.g., *Flavonifrator* in erythritol). However, when the inoculum effect was removed by testing 23 substrates with one faecal

sample, the medium explained 75% of community variation. The functional variation was explained by the medium, pH, and time (a cumulative effect of 43%).

We then investigated the compositional and functional shift between each enriched community, and observed a clear relationship between the functional and phylogenetic shifts after five backloppings. More specifically, the results showed that complex nutrients (e.g., polysaccharides such as pectin) had a greater effect on both the composition and function of the microbial community compared to intermediate metabolites (e.g., lactate). To investigate in more detail which metabolic pathways were used in each community, future research could use a denser sampling set-up (e.g., every 8 hours), in order to study the immediate community dynamics as well as transcriptomics to assign function to species more specifically[87,189]. Our study looked at communities after 48 hours, where nutrients and intermediate metabolites were possibly depleted, limiting our view on community dynamics[190]. Nevertheless, this finding highlights the importance of understanding the role of specific substrates in microbial metabolism, which is crucial to better understand the gut ecosystem.

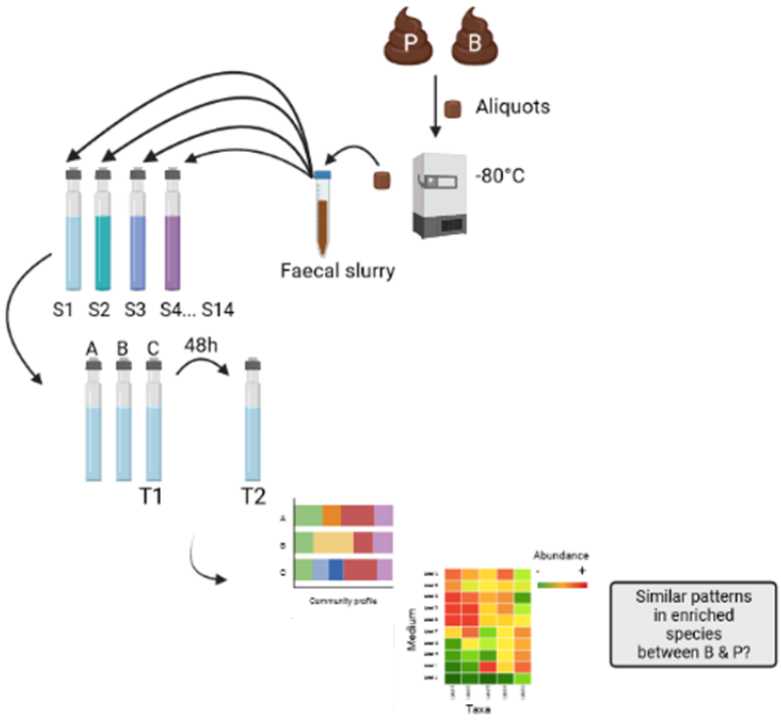
The time effect of repeated batch cultivation compared to LB only medium led to more divergent communities over time, suggesting that repeated exposure to a particular substrate can alter the microbial community structure and function and bring the community to specialise. However, the effect was not seen with all substrates, for many the community already specialised at the first fermentation timepoint (for example in mucin or maltose).

Conclusion

Overall, the findings of this study suggest that substrate but also pH, and time are key drivers of microbial community composition and function. By using self-assembled communities different microbial guilds can be observed which allows a deeper understand on community interactions from the gut ecosystem. In the future, using a wider ranger of donors could help unravel the true scale of interindividual variation[187,188,190] as well as exploring additional unknown guilds from the human gut microbiota.

Chapter VI

Substrate driven enrichment from two distinct enterotypes: Prev and Bact1


P
B
Aliquots
-80°C
Faecal slurry
S1 S2 S3 S4... S14
A B C
48h
T1
T2
Abundance
Medium
Taxa
Similar patterns in enriched species between B & P?

Abstract

The human gut microbiota has been stratified into several community types called enterotypes. Two of these, *Prevotella* and *Bacteroides* 1, are taxonomically distinct and can be associated with different dietary patterns. Understanding the impact of different nutrient sources on the composition and function of these communities is important for developing new biotherapeutics for microbiota-related disorders. In this study, we used substrate-driven enrichment to culture diverse bacterial communities from the *Prevotella* and *Bacteroides* 1 enterotypes in vitro, by testing fourteen different media on two faecal samples.

All bacterial communities were different in all media after culturing, with no trends observed in the beta-diversity. The individual signature was significantly different and led to significant differences not only in community composition but also in richness and individual enriched taxa. The repeated batch cultivation led to lowered richness in almost all the used media. However, some substrates like mucin and pectin induced high richness in both enterotypes, underlying possible cross-feeding interactions leading to this more diverse community. On the other hand, reinforced clostridial medium stimulated the growth of lactic acid producers, resulting in lower overall richness due to acidification of the culturing environment.

The different media affected the communities to a great extent, but the individual signature was still significant, indicating that individual differences should be taken into account when studying community interactions and dynamics in vitro. The observed cross-feeding interactions and selectivity suggest that substrate-driven enrichment can be useful for studying communities and understanding microbe-microbe interactions, as well as increasing the potential to isolate new taxa by enrichment. The results provide crucial knowledge of different gut-derived in vitro communities from nutritional preference of specific taxa to cross-feeding interactions.

Introduction

The human gut microbiota is important for the host well-being, and its composition and function have been the subject of growing research interest in recent years. The gut microbiota can be stratified into several distinct community types, or enterotypes, each of which is characterised by a specific set of dominant and co-occurring bacterial species. Previous studies have identified four main enterotypes: *Bacteroides* 1 (Bact1), *Bacteroides* 2 (Bact2), *Prevotella* (Prev), and *Ruminococcus* (Rum)[9,14,16]. The *Bacteroides* enterotypes (Bact1&2) are dominated by species of the *Bacteroides* genus. However, the Bact2 enterotype is characterised by a low bacterial count, lower diversity and fewer butyrate producers such as *Faecalibacterium prausnitzii*[15,16]. Bact2 has also been associated with a variety of diseases, including inflammatory bowel disease (IBD)[15] and depression[18]. The Prev enterotype is driven by the *Prevotella* genus and is inversely correlated with the abundance of *Bacteroides*[191]. Finally, the last enterotype is the most prevalent in the Western population and dominated by the Firmicutes phylum, mostly *Ruminococcaceae* family, this enterotype has the highest taxonomic richness[152].

While the Prev and Bact1 enterotypes are two distinct communities with distinct metabolic profiles[152,192,193], it is not yet clear how different nutrient sources impact the diversity and function of these communities. Moreover, little is known about the cross-feeding interactions within these enterotypes. In this study, we aimed to investigate the impact of a wide range of substrates on the community composition and function of the Prev and Bact1 enterotypes using repeated batch cultivation.

Specifically, we used two faecal samples, one from the Bact1 enterotype and the other from the Prev enterotype, and compared the communities derived from a variety of nutrient sources. By enriching gut bacteria using repeated batch cultivation, we aimed to better understand the factors that shape the gut microbiome interactions. The results of this study will provide new insights into the nutritional and cross-feeding interactions of the human gut microbiota and their potential implications for human health as well as the design of future biotherapeutics.

Methods

Sample collection and processing

The faecal samples were collected with collection kits[9,184] and stored at -80°C in the lab until processing. The cell density was measured by flow cytometry and the DNA was extracted as previously described[9] using the MagAttract PowerMicrobiome DNA/RNA kit (Qiagen, Germany).

Media preparation

For this study, fourteen different media were prepared. First, Luria-Bertani (LB) was made with a concentration of 10 g/L to be further used as the base medium, compared to the 20g/L recommended by the manufacturer. A stock solution of 10 g/L was prepared containing each separately one carbon source: trehalose, erythritol, acetate, propionate, succinate or lactate. These stock solutions were filter sterilised and preserved at 4°C until use. Later, the stock solutions were added to LB only to obtain a concentration of 1 g/L. Other media with non filtrable substrates (mucin, pectin and starch) were directly added with LB to be autoclaved. The commercial media that were used: RCM (reinforced clostridial medium), mGAM (modified Gifu anaerobic medium) and WC (Wilkins-Chalgren) were prepared according to the manufacturers. All the media were adjusted to pH 6.0 once filtered (0.2 μm) or autoclaved to obtain a sterile solution. More detailed information about the media can be found in Table 1.

Table 1: The 14 different media including substrates used in the media prepared with LB with each of the following carbon sources (grey) and from commercial media (white).

Substrate type	Medium	Details	Product reference
Baseline medium (LB)	LB	Base medium, contains peptone, NaCl, yeast extract	Luria Bertani broth Sigma-Aldrich
(poly)alcohol	Erythritol	Food additive	Sigma-Aldrich
Disaccharide	Trehalose	Dietary-derived molecule also used as food additive for frozen products	Sigma-Aldrich
Host-derived glycan	Mucin	Most abundant macro-molecule in mucin in the GI tract, source of nutrients for some bacteria	mucin from porcine stomach Type II Sigma-Aldrich
Microbial metabolite	Acetate	Intermediate molecule used in bacterial metabolism	Sodium acetate, Merck
Microbial metabolite	Hemin_Acetate	Acetate and hemin were added in the medium. Source of iron and intermediate molecule used in bacterial metabolism	50 mg of hemin (Sigma-Aldrich) was dissolved in 50 mL NaOH (Merck) 1M + sodium acetate Sigma-Aldrich
Microbial metabolite	Lactate_Acetate	Lactate and acetate were added in the medium. Intermediate molecule used in bacterial metabolism	sodium DL-lactate solution 60% w/w Sigma-Aldrich) + sodium acetate Sigma-Aldrich
Microbial metabolite	Propionate	Molecule produced by bacterial metabolism, important for colonocytes	Sodium propionate, Sigma-Aldrich
Microbial metabolite	Succinate	Intermediate molecule used in bacterial metabolism	sodium succinate dibasic hexahydrate Sigma-Aldrich
Polysaccharide	Citrus pectin (CP57)	Dietary molecule, CP57 57% degree of methyl esterification	LFT-SMART, KU Leuven, Belgium
Polysaccharide	Starch	Dietary molecule	starch from potato (soluble), Sigma-Aldrich, Germany
Commercial medium	mGAM & mGAM 1/10	Modified Gifu anaerobic medium Universal gut medium	Hyserve
Commercial medium	RCM	Reinforced clostridial medium	Oxoid, UK
Commercial medium	WC	Wilkins-Chalgren	Oxoid, UK

Inoculation and repeated batch cultivation

A faecal pellet was taken out of the freezer and was weighted and diluted to have a final concentration of 10^9 cells/mL. The sample was homogenised by vortexing in PBS until fully dissolved. Finally, the solution was filtered with a 0.5 μm filter to remove food debris. The inoculum was added in 1/100 concentration to each of the 14 prepared media in

triplicates previously reduced overnight in the anaerobic chamber in Hungate tubes. All the preparations for the inoculate and repeated batch cultivation were carried out in an anaerobic chamber at 37° under anaerobic conditions (10% H_2, 10% CO_2, 80% N_2) in a Don Whitley A35 Anaerobic Workstation with HEPA filter (Don Whitley Scientific, Shipley, UK). After the inoculation, the tubes were closed with a cap and mixed to homogenise. Then, they were incubated for 48 hours (timepoint 1). After 48 hours, 1 mL of the fermentation liquid was transferred to a new tube containing 9 mL fresh medium (preincubated overnight) for another 48 hours (timepoint 2). At that timepoint, 1 mL was taken for DNA extraction and the pH was measured (Figure 1).

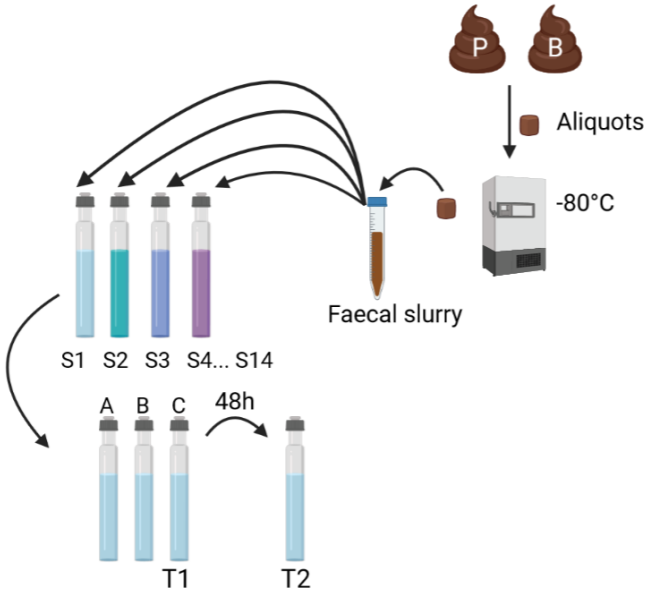


Figure 1. Experimental procedure, the faecal samples were aliquoted into pellets and kept at -80°C until processing. The aliquots were then diluted into PBS to have a final concentration of 10^9 cells.mL^{-1} in the faecal slurry to be inoculated in each medium after a 1/100 dilution. After 48h, 10% of the solution was transferred into fresh medium up to T2. Image made with Biorender.

Microbial community analysis

Fermentation samples were centrifuged, and supernatant was removed prior to storage at -80°C. Frozen faecal aliquots (between 150 and 200 mg) and fermentations were subjected to DNA extraction using the PowerMicrobiome RNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, USA) according to the manufacturer's instructions as described previously[9]. The V4 hypervariable region of the 16S rRNA gene was amplified using forward primer 515F (5' GTGYCAGCMGCCGCGGTAA 3') and the reverse primer 806R (5' GGACTACNVGGGTWTCTAAT 3'), modified with adapters and barcodes. Sequencing

was then performed using the Illumina MiSeq platform (MiSeq Reagent Kit v2, 500 cycles, 20% PhiX) according to the manufacturer's specifications to generate paired-end reads of 250 bases in length in each direction. After demultiplexing with sdm as part of the LotuS[132] pipeline without allowing for mismatches, fastq sequences were pre-processed using DADA2 pipeline v1.14.123. The taxonomy was assigned using GTDB release 202[135,166]. For relative microbiome analyses, each sample depth was downsized to 10,000 reads.

Statistical analysis

All statistical analyses and graphical representations were performed in R using the packages phyloseq[137] vegan[138] and ggplot2[140]. Observed richness was calculated with the R package phyloseq. Microbiome variation between individuals was visualised by PCoA using Bray-Curtis distances, only samples with more than 10,000 reads and amplicon sequence variants (ASV) with a relative abundance higher than 0.001 were included in the 16S data analysis. The contribution of metadata variables to the community variation was determined by distance-based redundancy analysis (dbRDA) on genus-level Aitchison distance (Bray-Curtis distances between samples after rarefication) with the capscale function in the vegan R package[138]. Correction for multiple testing (Benjamin–Hochberg procedure, FDR) was applied and significance was defined at FDR < 0.1. The cumulative contribution of metadata variables was determined by forward model selection on dbRDA with the ordiR2step function in vegan[138], with variables that showed a significant contribution to community variation in the previous step. Alpha-diversity, number of observed genera (richness) were determined using the vegan R package[138].

Results

Individual variation is driving community composition

Two faecal samples obtained from two distinct enterotypes, Bact1 and Prev, were inoculated into 14 different media in triplicate. The initial community composition of the two faecal sample differed (Figure 2A), with Bact1 (Ind B1) having high abundance of *Bacteroides/Phocaeicola* (5.1% and 13.2% respectively) and *Faecalibacterium* (10.9%), while Prev contained 53.6% of relative abundance of the genus *Prevotella* (Ind P).

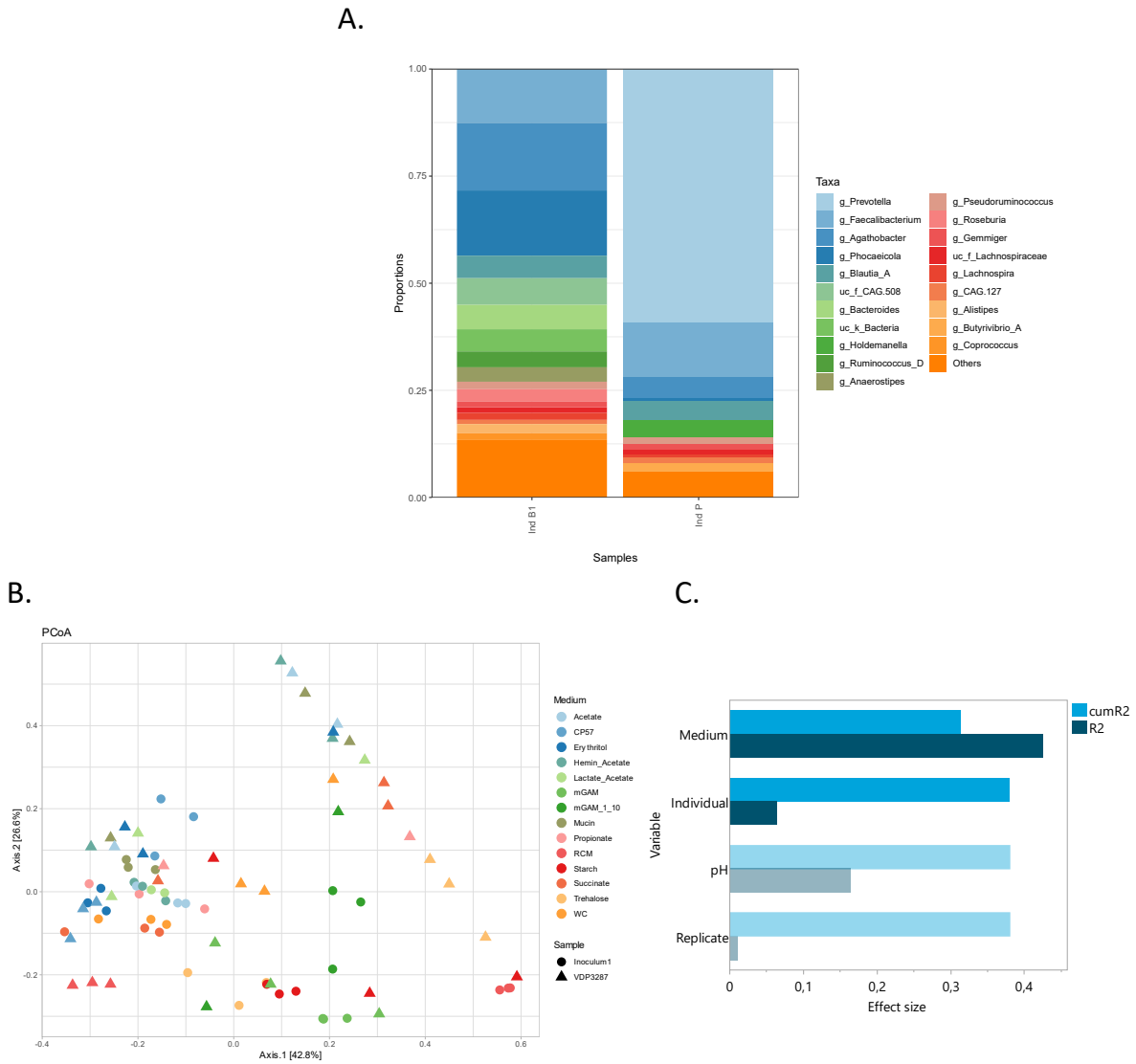


Figure 2. A. Relative abundances of the top 20 genera from the 2 selected faecal samples (Ind B1 and Ind P) derived from 16S rRNA gene community profiling B. C. Genus-level community variation of the fermentations in triplicates, represented by principal coordinates analysis (Bray–Curtis dissimilarity PCoA). Shown are samples from the different samples (shapes) and media (colours). C. Variables contributing most to compositional variation in the fermentations (dbRDA, genus level Bray-Curtis distances), either independently (univariate effect sizes) or in a multivariate model (cumulative effect sizes), medium and individual are two significant variables $p<0.05$.

After cultivation in the different media, the community composition was analysed at timepoint 2, i.e., after one step of repeated batch cultivation (two rounds of 48h incubations as detailed in the methods and Figure 1). This timepoint was chosen from the previous study described in Chapter 5, where a lot of communities were already distinct per substrate

compared to LB only and compared to each other. Analysis of the community variation showed that both the medium and the individual significantly explained about 40% of community variation cumulatively(mediumwith 31% and individual with an additional effect size of 7%, distance-based redundancy analysis, dbRDA, Figure 2 B and C).

Next, the results of the community composition for each medium per enterotype showed substantial differences between Bact1 and Prev community compositions as seen on Figure 3. For example, for acetate both communities had a high abundance of *Bacteroides* (20% relative abundance), however, the Prev community was also dominated by *Lactococuccus, Intestinimonas* and *Dorea* (total relative abundance of about 30%). Here again, it is clear that individual variation has a strong effect on community composition, as communities are very different while cultured in the same medium.

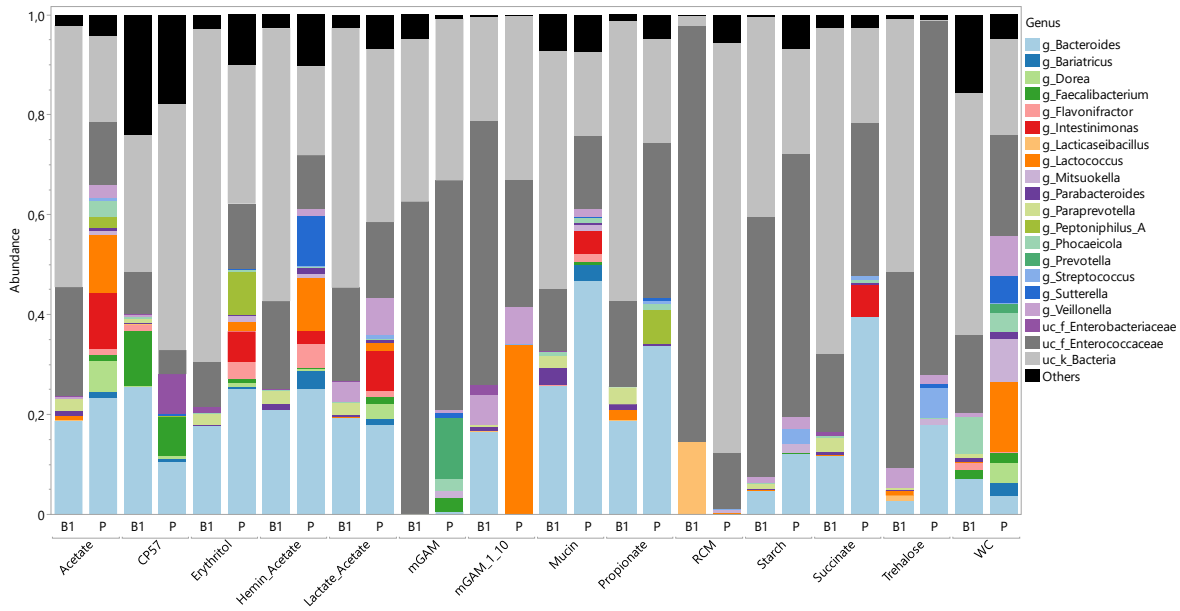


Figure 3. Relative abundances of the top 20 genera from the 2 individuals (Ind B1 and Ind P) fermented in each medium (average of the triplicates) derived from 16S rRNA gene community profiling per medium and per enterotype.

However, it is worth noting that while the Prev inoculum had a high relative abundance of the *Prevotella* genus (53.6%), this dominance was not observed in the in vitro community compositions (Figure 3). More strikingly, *Prevotella* was only detected in mGAM and WC media after culturing, while in other media it was not detected. In contrast, the *Bacteroides* genus was detected in most media of the Prev community (in light blue Figure 3), except

for RCM, although it was not detected in the faecal inoculum. The *Bacteroides* genus also became more abundant in the Bact1 community while in the faecal sample it was lower in abundance than *Phocaeicola* (5.1% of relative abundance for *Bacteroides* vs 13.2% for *Phocaeicola*). *Phocaeicola* was only detected, on average in all media at 1% of relative abundance. The results showed that the co-exclusion of *Prevotella* and *Bacteroides* genera, were seen in all media expect two, the two genera were found to co-exist in mGAM (with a higher relative abundance of *Prevotella* at 12.9%) and WC (with a higher abundance of *Bacteroides* at 4.2%).

Then, Bray-Curtis dissimilarity index for all the possible pairs between each set of triplicates was used to cluster the communities. The results indicated that most communities of the same enterotype clustered with each other rather than per medium (Figure 4). Only mGAM, mGAM1/10, starch, RCM and pectin (CP57) clustered by medium rather than enterotype. These results reinforce the observation that individual variation is a primary driver of differences in community composition.

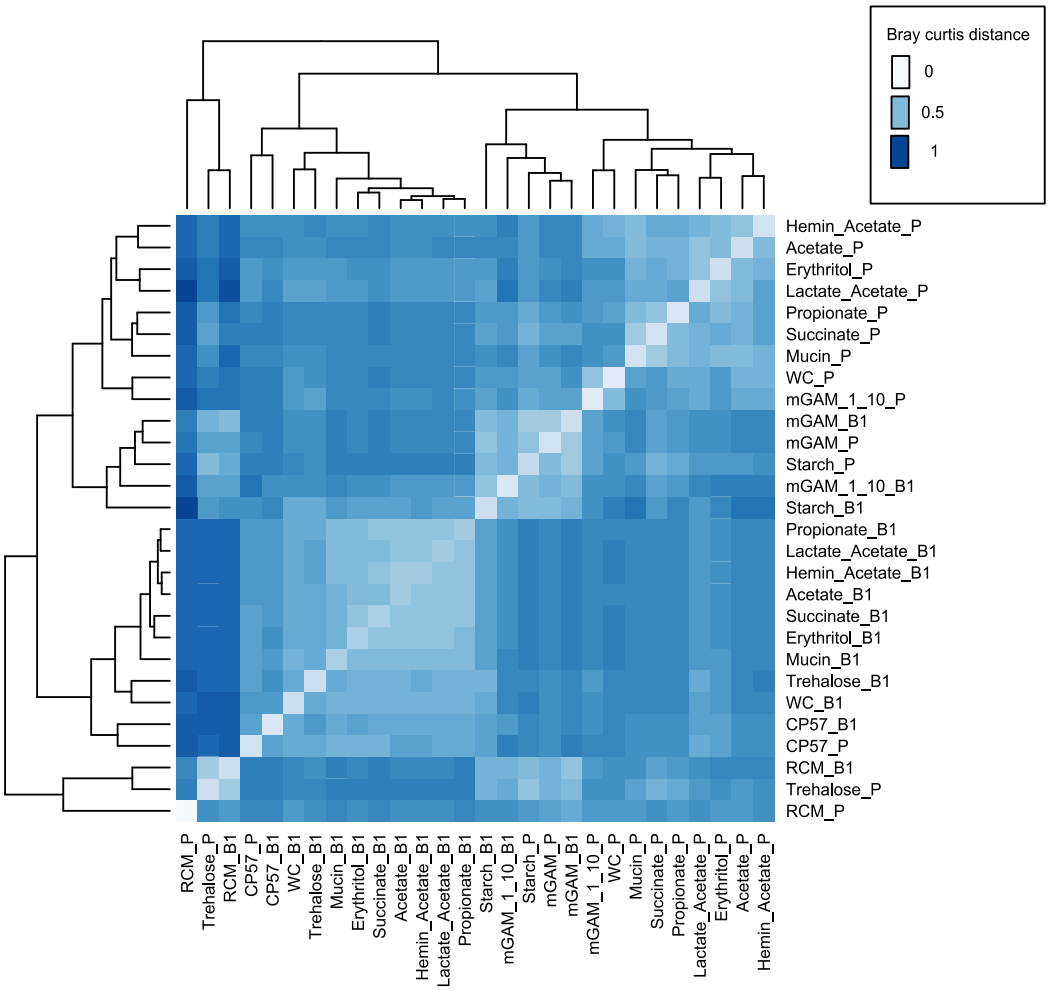


Figure 4. Bray-Curtis distances between all media from the two individual samples clustered by closest distances (average of the triplicates).

Individual variation affects richness and enriched taxa

The richness between the two enterotype-derived communities was found to be significantly different, with Prev having the highest richness overall (Kruskal-Wallis, $p < 0.01$). Only six media (mGAM 1/10, propionate, WC, trehalose, succinate and starch) did not show any significant differences between the two communities (Kruskal-Wallis, post hoc Dunn test $p < 0.05$, Figure 5). Overall, most backslopped Prev communities showed higher richness, although the original faecal sample had a lower richness compared to the Bact1 inoculum (104 vs 69 detected species with a relative abundance greater than 0.01%).

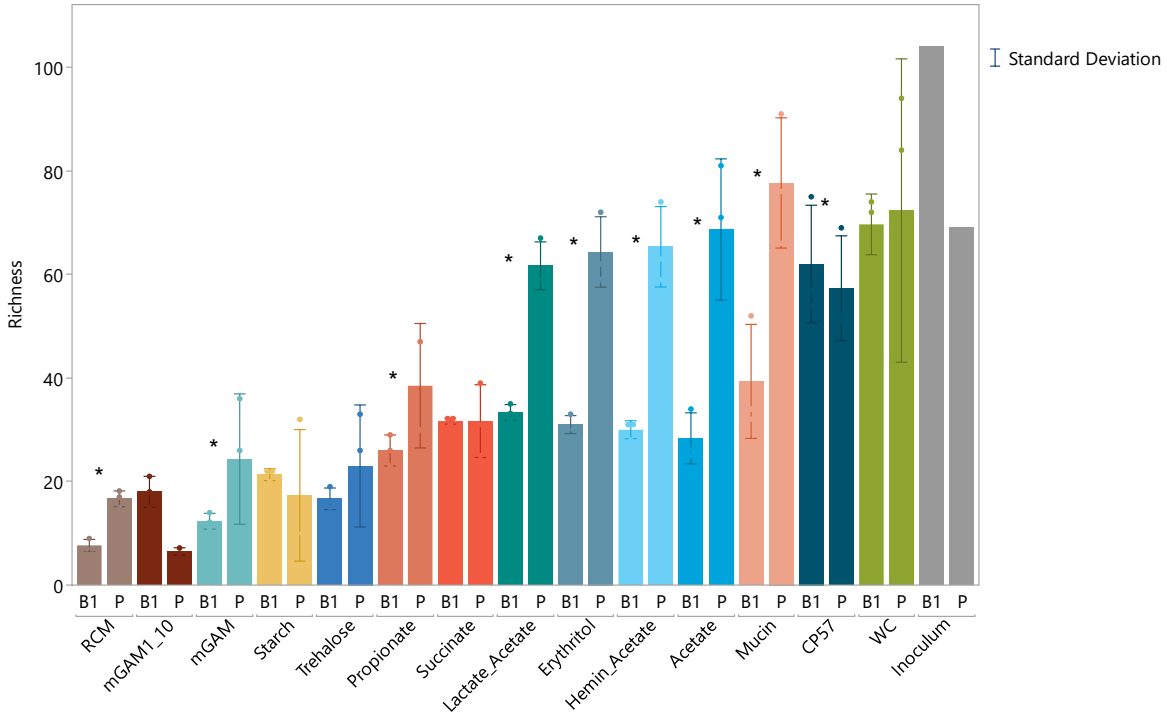


Figure 5. Mean richness at species level ordered by ascending values per medium and per enterotype, triplicates shown with standard deviation. On the right side, the richness from the stool samples can be found in grey. The star represents the significant difference between the Bact1 and Prev derived communities per medium.

Next, we looked at which media were enriching which taxa from each enterotype-derived community (Figure 6). *Bacteroides* was found in both enterotypes and most media, which could indicate generalist behaviour as it seemed to be able to utilise all substrates for growth. On the other hand, other genera, such as *Faecalibacterium* in mucin, were only found enriched in one enterotype here Prev, which could be due to the lack of a cross-feeding partner in this medium. Here again, these results highlight the significant impact of individual variation on the resulting community.

Nevertheless, it is worth noting that some genera were similarly enriched in the same medium in both enterotype-derived samples (e.g., *Faecalibacterium* and *Gemmiger* in pectin (CP57) or *Phocaeicola* in mucin). These results suggest that specific genera either preferentially use certain substrates and/or rely on cross-feeding with other enriched members. While at community level the resulting communities were different, specific taxa can be enriched in the same substrate. Enrichments can then be used for isolation campaigns regardless of initial community composition.

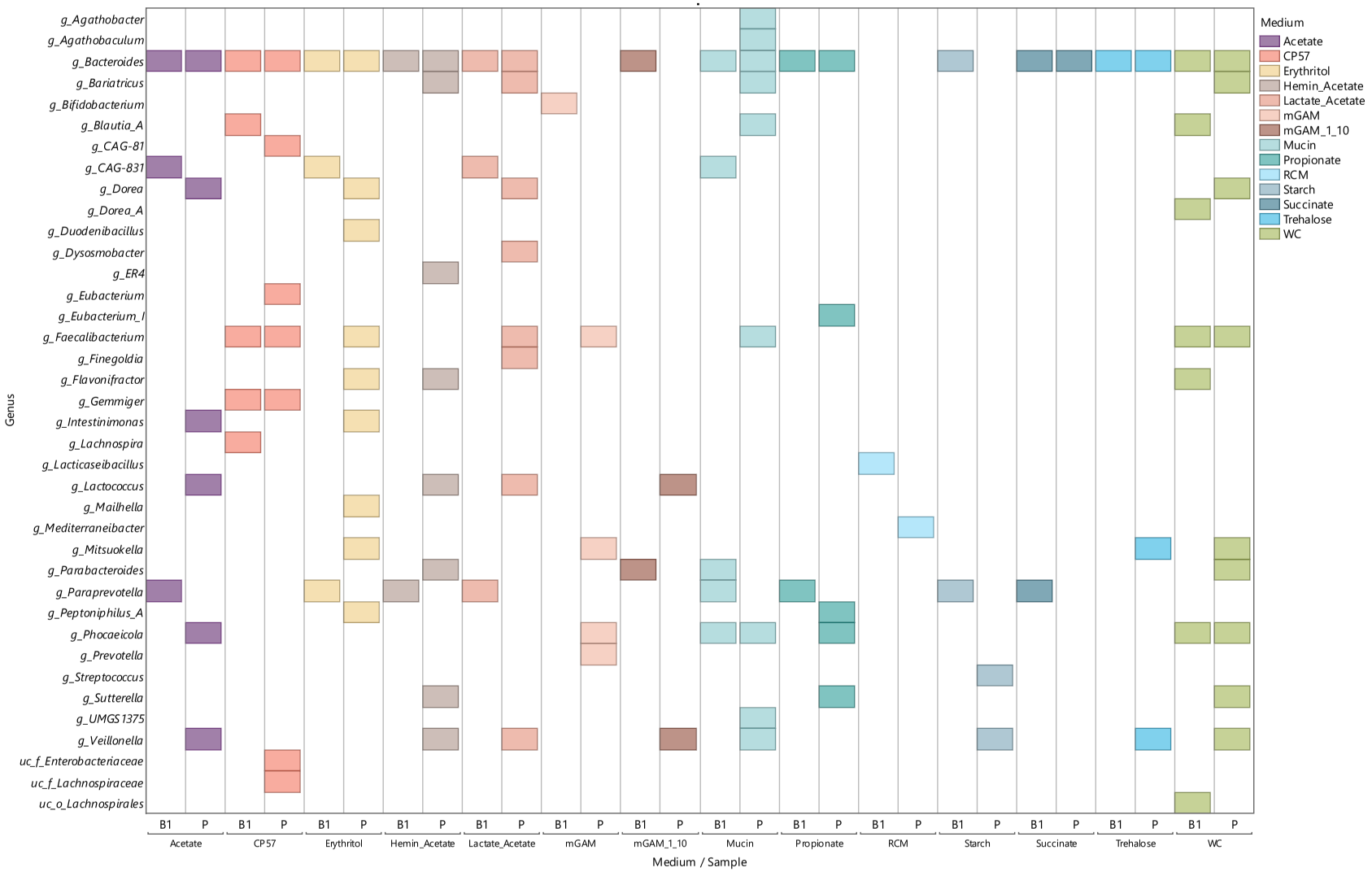


Figure 6. Presence - absence of each genus per enterotype per m.edium with a detection threshold at 1% relative abundance

Discussion

Studying community interactions in vitro is crucial for gaining a better understanding of the ecological dynamics of the human gut microbiota. In this study, substrate-driven enrichment was used to culture diverse bacterial communities from two distinct enterotypes, Prev and Bact1, using fourteen different media. A previous study compared these two enterotype comparing the fermentation of pea cell wall to fructooligosaccharides. They showed that the Prev sample had an increased fermentation rate and promoted the abundance of *Prevotella*, while Bact1 of *Bacteroides*[187].

In this study, we observed that all bacterial communities were different in all media after culturing. The beta-diversity analysis did not reveal any noticeable trends, such as the clustering of certain carbon sources (e.g., disaccharides) based on similar bacterial compositions. Overall, distinct communities were observed across all media, with approximately 30% of the variation in community composition explained by the media used. These results are consistent with prior reports indicating that the use of different media can drive differences in resulting bacterial communities[194,195].

Nevertheless, community variation was also considerably explained by individual variation, i.e., the initial community as previously seen in other studies[171]. As both initial faecal samples harboured very different community composition, the substrates alone were not able to lead to similar communities and the individual signature was still significant (RDA, $p < 0.05$). These differences led to significant differences in richness and in enriched taxa in the stool derived communities. Therefore, in order to further study community interactions and dynamics in vitro, individual differences must be taken into account in further studies. Using a wider range of donors could reduce these differences and help identify similar patterns in interactions. Nonetheless, not all taxa responded differently, and some such as *Faecalibacterium* or *Phocaeicola* were found to be enriched from both samples similarly in pectin and mucin respectively. More samples can be used in the future to further understand how individual species can be enriched in different substrates to better guide future isolation efforts.

For most media used, repeated batch cultivation lowered the richness as seen previously[196], although some media such as mucin and pectin, showed a high richness in both enterotypes. These substrates are complex molecules with different branched chains that can only be

metabolised by specific taxa and when broken down, the products can be further used by other species via cross-feeding interactions. These interactions could therefore be driving a more complex community encompassing several trophic levels. On the other hand, other media such as RCM stimulated the growth of lactic acid producers (e.g., *Lactocaseibacillus*). Therefore, due to the acidic environment, very few other species could survive lowering the overall richness in these samples. Moreover, it is important to note that all cultured communities differed from the inocula compositions. After culturing, about 1/3 of the genera enriched in the cultures (32.7% Bact1 and 40.3% Prev) were not detected in the inoculum as previously described[35,197]. On the other hand, about 65% of genera (67.8% for Bact1 and 62.6% for Prev) present in the inoculum were not detected in the cultures (Supplementary Table S6.1).

However, to truly capture interactions and dynamics, other -omics measurements would be necessary to disentangle the trends seen in this study. With both metabolomics and transcriptomics on top of community composition, also functional differences could be studied and would help to gain more knowledge on microbe-microbe interactions and community dynamics.

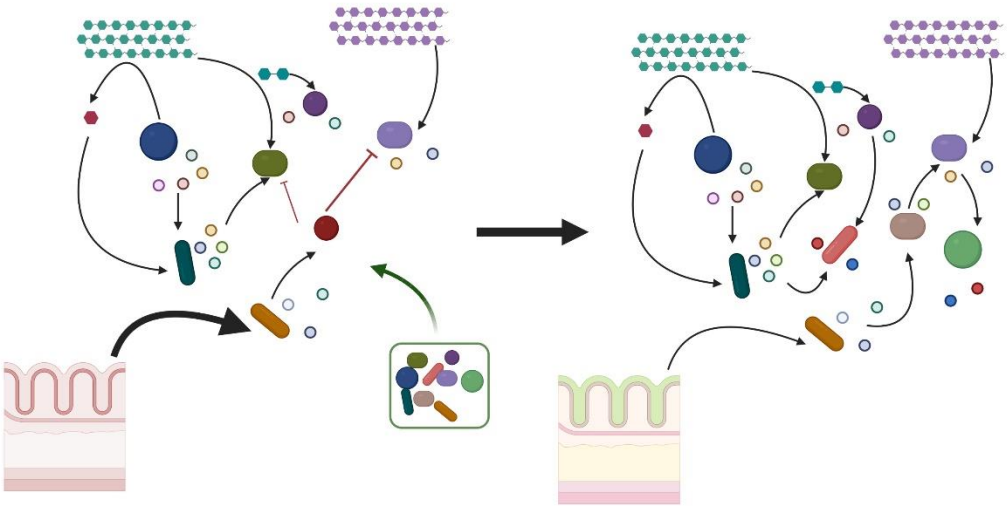
Conclusion

We observed important differences between the communities enriched from donor samples representing either Bact1 or Prev enterotypes. These results contribute to our understanding of in vitro community interactions and therefore could help designing more targeted biotherapeutics based on these enterotype differences. Future studies should focus on different enterotype-derived communities in order to deepen our understanding of the differences between each community types as well as using more donors to validate our findings.

Chapter VII
General discussion:
Towards designing next-generation probiotics from gut derived communities

Parts of this chapter have been published in:

Jorge F. Vázquez-Castellanos*, **Anaïs Biclot***, Gino Vrancken, Geert R.B. Huys, Jeroen Raes. Design of synthetic microbial consortia for gut microbiota modulation. *Curr Opin Pharmacol.* 2019 Dec. *contributed equally



The human gut microbiota is a highly diverse ecosystem consisting of trillions of microorganisms, including bacteria[8], viruses[27] and eukaryotes such as fungi[25]. These microorganisms have co-evolved with the human host and play a critical role in maintaining its health. The gut microbiota is involved in various functions for the host, including the breakdown of complex carbohydrates[152], the synthesis of vitamins[7] and essential amino acids, and the development and modulation of the immune system[48]. Additionally, the gut microbiota interacts with the nervous system and has been implicated in brain function and behaviour[198].

Dysbiosis, referred to as an imbalance in the gut microbiota, has been linked to several diseases, including inflammatory bowel disease[63,64], type 2 diabetes[66], and colorectal cancer[65]. In recent years, advances in culture-independent techniques such as next-generation sequencing have provided a more comprehensive view of the composition of the gut microbiota. However, despite these advances, our understanding of how the different bacterial species interact with each other in the human gut and how these interactions influence the overall function of the community is still limited. Recent research has highlighted the importance of microbial interactions in shaping the gut microbiota and its functions[199]. Studies have shown that different species of bacteria can interact in various ways, such as through competition for resources as well as via cross-feeding[2,117,200]. These interactions can impact the composition and function of the gut microbiota and may contribute to disease development or prevention. Moreover, acquiring knowledge about the gut communities will allow the translation of microbiota research to design novel biotherapeutics.

However, we need to first better comprehend these interactions which is challenging due to their intricate interactions and large diversity present in the human gut ecosystem. To better understand these interactions, simplified communities can provide insight into how different bacterial strains coexist within the gut environment. Such simplified communities can be created using two different approaches: a bottom-up approach and a top-down approach. In a bottom-up approach, individually selected bacterial strains are assembled into a community, while in a top-down approach, a complex community is reduced to a simpler one using culture-based enrichment of a faecal sample.

The primary objective of this thesis is to build a conceptual and methodological framework that could serve both the fundamental understanding of microbiota interactions but also to use simplified communities towards designing next-generation probiotics. The thesis is structured into five research chapters, each addressing a different aspect of this objective.

Building defined communities using an iterative methodology ("bottom-up" approach)

Selection of strains

The bottom-up approach involves selecting individual bacterial strains that are thought to be important to the gut microbiota and combining them into a simplified community. This approach allows to control the composition of the community and study the interactions between specific bacterial strains in isolation. Depending on the end goal of these defined communities, either for a fundamental approach, i.e., studying interactions in the gut ecosystem, or for an applied goal, i.e., using a defined community to restore a dysbiotic microbiota, the selection approach will differ. The use of defined communities to study the gut ecosystem is a recent approach, and most studies so far are focusing on core taxa[10,164]. These core taxa represent therefore the main species found in most individuals in human populations, usually from a Western population. In this thesis, a novel strategy was used to design defined communities. The study focused on designing health-associated enterotype functional cores (HAEFC) based on enterotypes and the metabolic capabilities of the taxa, in addition to considering their prevalence and abundance in healthy individuals (**Chapter 3**). The study also performed full metabolic characterization of each selected species using both in silico and in vitro data and identified functional clusters within the cores. Our results provide insights into the ecological niche of the core species and potential competition and cooperation at the community level, which can be used for the in vitro assembly of the core communities. Future studies could work on more clinically oriented defined communities to target specific missing species in a diseased state using information gathered on cohort studies in order to restore a more balanced ecosystem. The community could also be designed with a basic core community at its centre with additional species tailored to a specific individual or disease.

In silico identification of core community members also revealed taxa that are underrepresented in public culture collections or even lack culturable representatives from

human gut origin. Therefore, to diversify future assemblies of defined communities, new isolation campaigns are needed to increase the number of cultured species from the human gut[105]. In **Chapter 2**, faecal samples of 11 healthy human volunteers from a Western population were cryopreserved under different conditions in order to find the optimal faecal sample preservation, essential step to safeguard the viability of the broadest taxonomic diversity range possible. Analyses of compositional variation showed that cryopreservation medium conditions affected quantitative recovery but not the overall community composition of cultured fractions. This work provides insights into how to build a comprehensive culture collection of gut microbiota members. The in-house culture collection allows a better knowledge of strain history with extensive donor metadata as well as a tailored collection relevant to the research questions to define communities compared to purchased strains from public collections.

However, the choice of culture medium is crucial for comparing bacterial recovery across preservation conditions. The medium used (mGAM) favoured the growth of a few specific taxa, such as *Bifidobacterium, Bacteroides*, and *Phocaeicola*, while other highly abundant gut members remained undetected in the cultures (e.g., *Faecalibacterium*). These strains may have more specific nutritional needs, and/or might have been outcompeted by fast growing strain. Additionally, the sample dilution rate co-determined the species richness recovered during isolation, which can be especially relevant for low-abundant species. Agar-based isolation approaches have limitations, such as the overgrowth of slow-growing species by faster-growing ones and the inability to pick microscopically small colonies. Therefore, single-cell-based strategies are emerging as an alternative approach that suffers much less from these limitations[106].

For the bottom-up design of artificial communities, previous studies have either looked at identifying specific taxa or designing the community to produce specific compounds (e.g., propionate[150]). However, few studies have considered both compositional and functional cores[151], and to our knowledge, none have investigated these in terms of enterotype-based microbiome types. In this thesis (**Chapter 3**), core members of the microbiota and their metabolic capabilities were identified in silico using a previously generated dataset of 1,106 subjects participating in the Flemish Gut Flora Project[9]. The aim was to select bacterial taxa

that could be assembled to form health-associated functional core consortia. In this study, we focused on the community types with a dominance of either *Prevotella* or *Bacteroides* namely the Prev and Bact1 enterotypes respectively. The main rationale for this choice is based on the opposite trade-offs displayed by these two genera in the human gut ecosystem[201] , as supported by the observation that most individuals have only one of both genera dominating their community composition. The selection of predicted species was based on their prevalence in the population as well as their key metabolic properties. The results of this work provided a foundation for the selection of bacterial members for further in vitro experiments (**Chapter 4**).

To study the functional profile of the cores in depth, a metabolic ID was performed for each selected species, and three functional clusters within the cores were defined using spearman correlations of genomes KO annotations from type strains using the IMG database. The results of this analysis greatly depend on the quality of genomes and annotations and should be taken into account when comparing in silico to in vitro results. The results showed a high level of redundancy within the cores, particularly in cluster 1, comprising many substrate degraders, which could lead to increased competition within the community in vitro. Conversely, potential cooperation was also observed using reverse ecology analysis, with several highly cooperative species identified in the community. These results highlight the importance of taking into account potential competition when designing in vitro communities, and the potential for cooperation within the gut microbiota. The cores were then defined as health-associated enterotype functional cores, representing the Bact1 and Prev enterotypes.

Defined communities: from monocultures to consortia

In this thesis, a defined community was assembled in an iterative approach which added more knowledge on bacterial interactions (**Chapter 4**). Before assembling the community, the selected species (from **Chapter 3**) were tested in vitro to assess their preferred substrates and the resulting metabolic output. These results allowed to design a suitable medium for all members of the community. Beyond metabolic characterizations, additional information could have also been gathered from determining growth rates which could add valuable information on potential resource and spatial competition. Preliminary data on

growth rates for some community members used in this study showed a disparity between lag phases, ranging from 0 to 8 hours in mGAM, with *Phocaeicola vulgatus* where no lag phase was recorded in the tested conditions (Figure 1). This result could explain the dominance of *Pho. vulgatus* observed in the fermentation overtime, suggesting that *Pho. vulgatus* has a competitive advantage over other species in the community, potentially due to its ability to rapidly utilise available resources and occupy spatial niches.

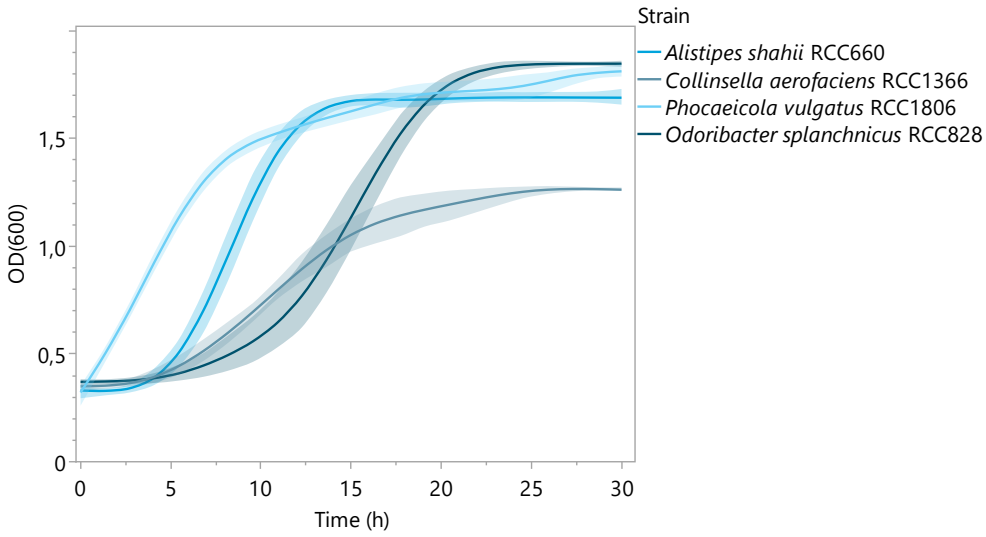


Figure 1. Growth rates (OD_{600}) for different strains grown in mGAM (with deviation shown from the triplicates), methods in Supplementary material.

More information about the growth rates of each species could give more insight on potential growth competition in the chosen medium. It is important to note that these growth rates are medium dependent and should therefore be tested in the final medium (in this case MM++) used to co-culture the community.

Next, to better understand and predict interactions, pairwise co-culturing experiments were performed. This setup allows assessing competition and cooperation on a 1-to-1 basis and therefore adds valuable knowledge on microbe-microbe interactions. In this thesis, we aimed to exclude spatial competition and growth rates and instead focus on nutrient competition and cooperation. For this purpose, we decided to use a method that would physically separate the tested species. One possible method could have been the use of spent medium. Here, isolates are grown separately, and individual supernatants are filtered and used as spent culture medium to grow another member of the community. However,

due to the presence of a high number of large molecules in the medium (e.g., mucin, starch, pectin, and xylan) that are not entirely soluble, filter sterilization of MM++ medium was not feasible. Therefore, we employed transwell plates as an alternative approach. This method enabled to physically separate the two isolates and study competition and cooperation through growth measured as cell count after 24 hours. Species exhibiting increased growth (>0.5 $\log_{10}$ compared to their growth in monoculture) when paired with another species were considered to cooperate, while those showing decreased growth (<0.5 $\log_{10}$ compared to their growth in monoculture) were considered to compete. Comparison of in vitro data and in silico predictions revealed that both *Collinsella* and *Dorea* showed high cooperation with members of the community, as well as confirming earlier observations supporting the role of *Ruminococcus bromii* as a key member of the community as seen in previous studies[38]. However, the two former taxa are not well studied, and little is known about their metabolism and possible cross-feeding interactions within the gut ecosystem. Further research will be necessary to better understand their role in the community. And while co-cultures have been reported to not predict community interactions when comparing them to complex communities[173,174], they still provide valuable knowledge to the field. Indeed, they can highlight key interactions that can be more easily modelled and enable to uncover important ecological principles that can be applied to more complex systems. One example is the cross-feeding interaction between lactic acid producers (e.g., *Bifidobacterium*) and *Faecalibacterium*[39] as reported previously using co-cultures.

To further study interactions, the next step was to use small communities (~ 4 to 5 species). The first approach in this thesis was to assemble the first functional group, mainly substrate degraders (all part of functional cluster 1, **Chapter 3**). However, the community became dominated by a few taxa (mainly *Phocaeicola*) while most species including *Odoribacter* and *Alistipes* were already undetected after 24 hours. This was probably due to increased competition between substrate degraders as many have very close functionality. This observation led us to take the opposite approach, i.e., to assemble small communities that represent different trophic levels. Briefly, two substrate degraders (the enterotype driver coupled to a species that was not found in any functional cluster such as *Ruminococcus bromii*) were chosen with one member of cluster 2 and one member of cluster 3. Briefly, cluster 2 was composed of taxa that could degrade a limited number of substrates and produced

several intermediate products (e.g., lactate), while cluster 3 was composed of butyrate producers (e.g., *Faecalibacterium*). These communities contained all the functional capabilities with limited redundancy. Two communities were designed for Bact1 and Prev enterotypes to be tested in vitro. The results showed clearly that *Pho. vulgatus*, while co-existing with the other species, was still dominating the community up to 85% of relative abundance. Therefore, although the fast growth effect observed above could be mitigated to some extent by reducing redundancy, it remained an important determinator of the resulting community composition.

Nevertheless, all members were able to co-exist for the duration of the experiment which proved to be of key value to generate predictive results towards the successful assembly of the full communities in the following experiment. Furthermore, the importance of different functions was highlighted when the enterotype-driving species in the community were replaced by other species of the same or a related genus, i.e. *Phoceicola vulgatus* by *Bacteroides uniformis* and *Bacteroides thetaiotaomicron,* and *Prevotella copri* by *Prevotella lascolaii*. This showed that functional capabilities are more important within the community, and that different sets of species resulted in the same community, where all species could co-exist with similar relative abundance. This could be tested further by replacing other species in the community as well as investigating the functional output of the different communities as it was not done in this study.

Culturing a defined community: which parameters are important?

The first step to grow a defined community is to find a suitable medium for all of its members. Most studies do not specify why they used the medium in their study[202], while others use systems designed to resemble the gut environment such as the SHIME system[150]. In this thesis, the results from **Chapter 4** showed that both in silico prediction of substrate use and their validation in vitro were useful to design a suitable medium for the community while adding knowledge into the metabolic preferences of these core species. While only a limited set of substrates were used in the panel mostly including carbohydrates (i.e., starch and pectin) and microbial metabolites (i.e., acetate or lactate), simpler sugars such as fructose, could also be tested. Other gut molecules such as amino-acids, lipids, as well as bile acids or vitamins could also be included to investigate the full metabolic preferences of the different species. Substrate is an important factor to define community composition as

also seen in the top-down approach (**Chapter 5 & 6**) where polysaccharides were shown to lead to a diverse community, while more simple molecules (monosaccharides and microbial metabolites) led to less rich communities and a strong enrichment of specific species such as lactic acid bacteria in trehalose. Designing a suitable medium for the desired output, either community composition or functional output, is therefore a crucial step into designing defined communities for clinical applications.

Apart from the medium, starting pH and pH control during fermentation also showed to have an impact on community composition. Both in the bottom-up (**Chapter 4**) and top-down (**Chapter 5**) approaches, communities were different at different pH values. In the latter case, for example, *Akkermansia* was not detected in some communities probably due to a too low pH. Previous studies have established a correlation between colon pH and human health in vivo, showing that maintaining a near-to neutral pH level was critical for good health[203]. An increase in faecal pH was observed to cause significant changes in the composition of gut microbiota, mainly in the abundance of *Bacteroidaceae*, *Bifidobacterium,* and *Enterobacteriaceae*. At the functional level, the levels of acetic, propionic and butyric acid were also altered in favour of higher lactic acid levels[171,203,204]. Several in vitro analyses provided further evidence that *Bacteroides* was sensitive to acidic pH conditions, while taxa from the Firmicutes phylum were more tolerant as also seen in our study[204]. In the literature, the results of in vitro studies corroborate the observations derived from the in vivo analysis and offer valuable insight into the interplay between pH, the gut microbiota, and health. Nonetheless, a significant knowledge gap persists with regard to the effects of pH on most members of the the gut microbiota, whether or not in combination of the growth promoting or inhibitory effects of the metabolites produced at the given pH condition. The pH value of the growth environment constitutes an essential parameter for in vitro studies and should be considered while cultivating defined microbial communities. Further research is required to understand how pH affects the interactions among microbial communities, given that colon pH ranges from 5.2 to 7.0, implying the presence of diverse microniches with specific pH values in vivo.

Another parameter, the relative abundance of bacterial species introduced into a community, also known as the input ratio, is often overlooked in community assembly

studies but could significantly affect the diversity and composition of the resulting community. Competitive interactions for nutrients and space between introduced species can be influenced by their initial relative abundance, which in turn may shift the community composition. A 1:1 input ratio is commonly used in current studies[150,151], and to our knowledge only one study investigated the impact of different input ratios on bacterial community assembly experiments[205]. More specifically, Kurt and co-workers compared equal ratios to "controlled ratios" aiming to mimic the natural abundance of the respective genera. Their results showed that overtime (after 13 days), in a continuous fermentation both communities converged to a similar state. Depending on the setting of the experiment (time, fermentation setup and set of species studied), different input ratios should therefore be considered to study community dynamics and interactions. In this thesis, we examined both equal (1:1) and 16S ratios, i.e., a ratio based on the relative abundance of species in cohort studies (here FGFP[9]), and found no significant difference in community richness between the two ratios (Figure 2). Nevertheless, future studies should not overlook this variable when assembling a defined community as it could be dependent on the different species and the fermentation setup used (e.g. batch and fed-batch used in this study compared to continuous fermentation as used in Kurt et al[205]).

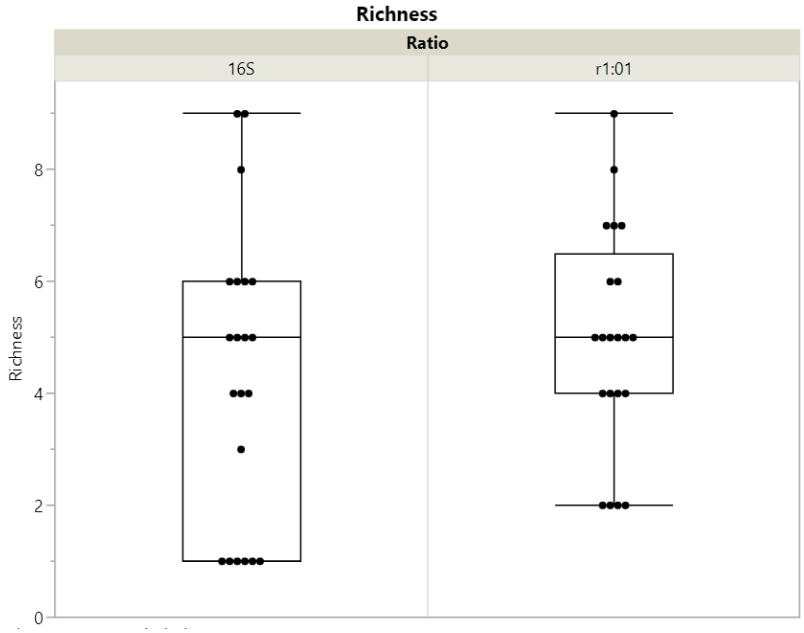


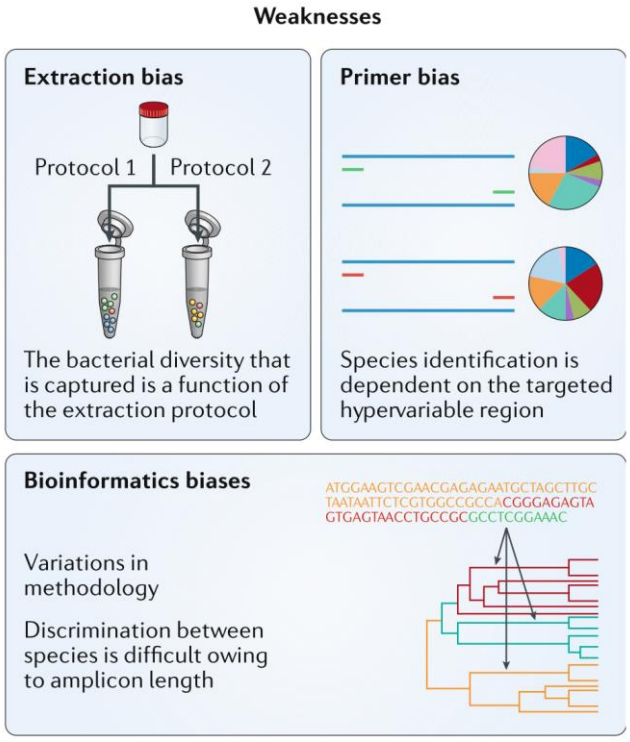
Figure 2. Richness of 1:1 vs 16S ratio communities using two communities at different timepoints with either 1:1 of 16S starting ratio (n=20), methods in Supplementary material.

Revisiting techniques to study community composition

During the course of this thesis, multiple techniques were employed and compared to study microbial community compositions of the HAEFCs in **Chapter 4**. While sequence-based approaches allow to study community compositions with a throughput and phylogenetic coverage culture-based techniques cannot, one has to keep in mind that several biases can be introduced during the sequencing process. From the methodological point of view, DNA extraction, library preparation and sequencing are all major steps in the pipeline during which biases can be introduced. For example, differences in DNA extraction efficiency can lead to variations in the yield and quality of DNA obtained from different samples[206,207]. Similarly, differences in library preparation methods can affect the representation of different microbial taxa in the final sequencing data[208], especially in the PCR step during library preparation for 16S rRNA gene sequencing where some bacteria can be preferentially amplified[209,210].

Additionally, computational biases arising from bioinformatics pipelines used for data analysis, e.g., different methods for quality control and filtering of sequencing reads, can affect the final composition of the sequencing data. Finally, differences in taxonomic classification methods and reference databases used for profiling can also introduce computational biases[208] (Figure 3A). These biases can significantly impact the accuracy and reproducibility of studying bacterial communities. Therefore, it is important for future studies to carefully consider these potential sources of bias when designing their experiments and analysing community composition, especially when studying small communities where such biases could be aggravated due to the low diversity.

A.



B.

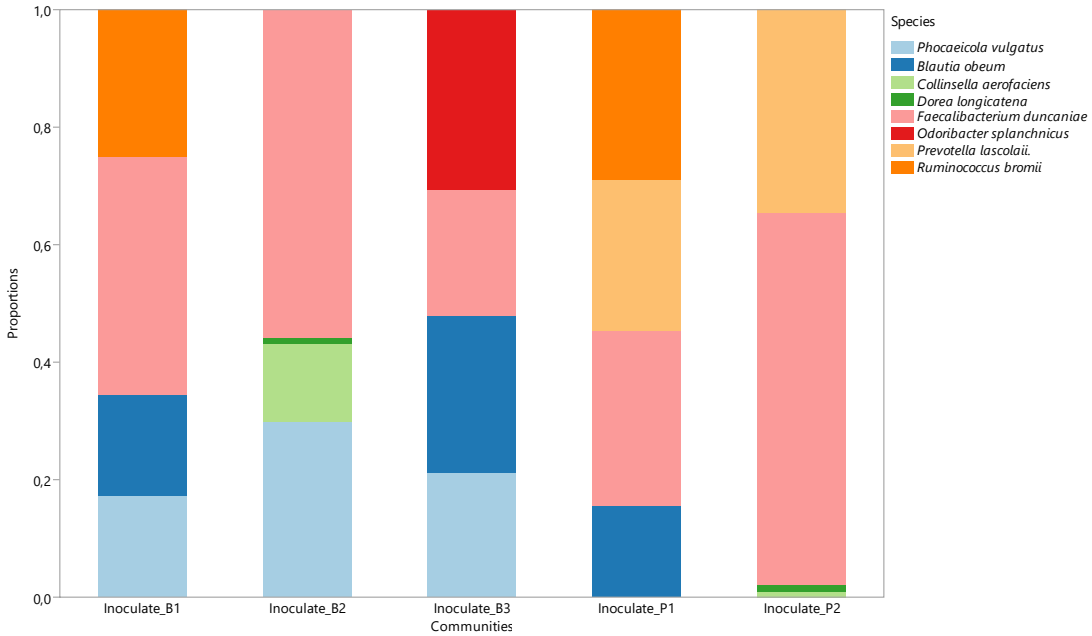


Figure 3. A. Weaknesses of culture-independent methods for community profiling from Lagier et al.[34] B. Community composition of five artificial inocula (three from Bact1 community, B1, B2 and B3, and two from Prev community, P1 and P2, from Chapter 3) with 1:1 input ratios showing important variability (16S rRNA gene sequencing with MiSeq), methods in Supplementary material.

In this thesis, the community profiles of the inoculates of the four-member communities showed discrepancies with 16S rRNA gene sequencing using MiSeq.. The resulting community composition did not reflect the 1:1 ratio (Figure 3B), with mostly *Collinsella* and *Dorea* being underestimated (light green and green respectively). Likely, these discrepancies could be attributed to biases introduced during extraction, PCR amplification of the 16S rRNA gene, and bioinformatic processing. Although developing an optimised protocol suitable for most gut bacteria to mitigate extraction bias would be challenging, we assessed whether simply removing the PCR step would make a substantial difference.

Oxford Nanopore Technologies (ONT) offers a next-generation long-read sequencing platform that can be used to sequence whole genomes from bacterial cultures and communities, and that allows to bypass the PCR step. By using different sets of co-cultures with a 1:1 ratio for the species used in this thesis, preliminary results showed that abundances determined by ONT sequencing were closer to the expected abundance for many species (Figure 4A). The results were not significantly correlated with their Gram-type or genome length, although these parameters did have an effect. To gain a more comprehensive understanding of the potential influence of these factors, further evaluation involving a larger number of different species would be needed.

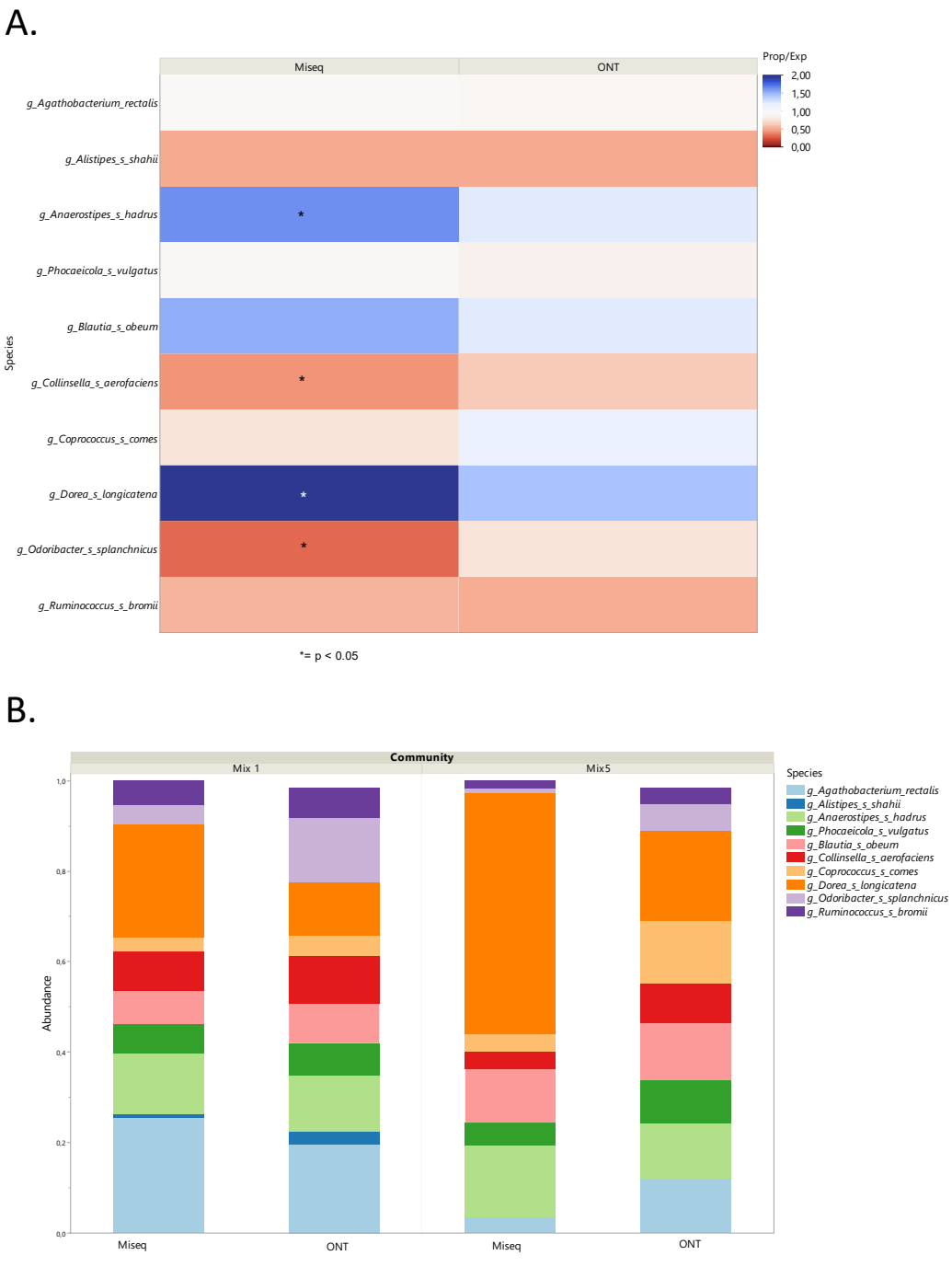


Figure 4. A. Ratio of the expected vs measured proportion from 36 DNA mixes for each species. Expected proportions are the 1:1 ratio from the inoculated co-cultures. Results closer to 1 (white) indicate that the proportions are close to expected values. Significance between MiSeq and ONT is indicated by a star. B. Community composition analysed from 16S rRNA gene sequencing (MiSeq) and Oxford Nanopore Technologies (ONT) sequencing of identical technical replicates for the two communities studied in the thesis: Bact1-like M-HAEFC (Mix 1) and Prev-like M-HAEFC (Mix5), methods in Supplementary material.

A follow-up project was then set up to optimise the bioinformatics pipeline to further remove the biases at this step (Simcock, Biclot et. al. *unpublished*). Different classifiers will be tested to assess the difference in community composition using both long and short read classifiers, a pipeline parameter know to have impact community profiles[211]. Nonetheless, already removing the PCR step allowed a better estimation of community profiles (Figure 4B), which led to the use of this method in this thesis.

To compliment sequence-based approaches, we also evaluated the CellScanner tool which relies on flow cytometry data. This tool performs gating and uses supervised classification techniques to assign events from communities to species defined by the user. This method avoids labour-intensive DNA extraction and, in contrast to 16S rRNA gene sequencing, generates absolute abundances. In a previous study, the method performed equally well or better than 16S rRNA gene sequencing in two-species cocultures and in a four-species community[168]. In this thesis, CellScanner was used or in the specific case where samples yielded an insufficient number of reads to be processed with ONT sequencing. Since this method does not rely on DNA as information molecule, it also is not subjected to the potential biases. Beyond the scope of this thesis, future work could compare CellScanner to ONT in order to evaluate if community profiles from CellScanner are still superior to this sequencing technique, as it was already shown to perform at least as well as 16S rRNA gene sequencing[168]. Despite its advantages, it is important to note that CellScanner has not been tested on communities containing more than four taxa. In addition, cell size diversity and cell cycle variations may introduce unwanted variation in some bacterial taxa)[168,212]. Such variation can have an impact on the comparability of monoculture data obtained from different experiments and should therefore be taken into account when using the tool.

The community profiling method used to study defined communities is of great importance as it determines the accuracy of the data obtained. A few of these methods were tested in this thesis and were shown to each come with its own strengths and limitations. In our hands, the use of CellScanner (for very simple communities) and long read sequencing using the ONT platform were preferred to the gold standard 16S rRNA gene sequencing.

Self-assembled communities ("top-down" approach)

In **Chapters 3 and 4**, the focus of this thesis was to assemble communities from the bottom up. However, this approach can be very challenging as isolates originating from different donors might be ecologically incompatible the design of a suitable medium allowing growth of all constituting species can be a long and complex process. This part of the thesis came with a lot of trial and error work related to the identification of suitable medium and culturing conditions. As discussed earlier, many parameters should be taken into account given that a change in substrate or pH can drastically alter community composition and function.

Alternatively, **Chapters 5 and 6** investigated the top-down approach to study bacterial interactions in simplified gut communities. The use of different substrates for enrichment as well as repeated batch cultivation forced the communities to specialise. One of the major findings of this part of the project was that individual variation (i.e., different donors) explained most of the variation generated during fermentations whether the samples were originally quite similar in composition i.e., belonging to the same enterotype (**Chapter 5**) or very distinct i.e., belonging to different enterotypes (**Chapter 6**). However, this effect was mostly observed at the level of the overall community composition, as specific enrichment of taxa was observed under the same medium from different donors such as *Flavonifrator* in the presence of erythritol, or *Faecalibacterium* when including pectin as substrate. Therefore, this suggests that enrichment of specific species is possible regardless of the initial composition of the donor, for healthy volunteers. By testing a wide range of substrates encompassing different trophic levels of the gut environment i.e., complex molecules degradation (e.g., cellulose), simple sugar degradation (e.g., maltose) and fermentation intermediates (e.g., lactate), we showed that with more complex molecules (i.e., polysaccharides including pectin and cellulose), communities were highly diverse while simpler molecules led to more enriched communities, less diverse but with higher increase of specific taxa (e.g., lactate producers in starch).

Other communities showed to be very functionally distinct upon nutrient intervention. On example is the community enriched in the presence of starch, with high accumulation of lactate, acetate, formate, and glucose compared to other media. Possibly, this was due to

the presence of lactate producers that acidified the medium leading to a reduced overall diversity. Therefore, accumulation of certain metabolites could be due to the lack of consumers present in the community. Beyond the scope of this thesis, additional omics methods such as transcriptomics would give a more in-depth view on individual metabolic roles in the community. In addition, an increase of the frequency of sampling could add more resolution to data analysis. In this study, samples were acquired after 48 hours at each repeated batch cultivation step. It can be expected that a denser sampling setup, for example every 8 hours, could bring more insight into the dynamics with the community overtime.

In summary, the top-down approach showed that two main types of communities can be enriched. On the one hand, diverse communities encompassing different trophic levels present in the gut ecosystem (i.e., complex substrate degraders to butyrate producers) can be obtained with less accumulation of specific metabolites. On the other hand, smaller communities can arise with more specifically enriched taxa and strong functional effects due to accumulating metabolites. The strategy used in Chapter 5 and 6 originally intended to isolate small communities specialised for a specific task. Here, we showed that this is only possible for less complex molecules such as mono- or disaccharides as well as intermediate microbial metabolites such as lactate. On the other hand, the observation that under complex substrates each replicate results in the same complex community suggests that the presence of the whole community to degrade these compounds is the ecologically more favourable scenario.

Studying gut communities: what we learned

Bottom-up approaches focus on understanding the mechanisms that give rise to functional properties of systems by studying the interactions between the components of the system. These strategies have led to significant insights in the properties and interactive capacities of single species which are relevant for applications in the development of new biotherapeutics and for metabolic engineering of microbial strains. This way, novel microbial functions and metabolic pathways can be discovered, which ultimately provides a more comprehensive understanding of the microbiome. However, these approaches need iterations and can therefore be time-consuming and may not capture the complex

interactions between microbial communities. In this thesis, the bottom-up approach allowed to characterise more in-depth the metabolic profiles of each member of the community as well as studying interactions at different complexity levels (i.e., co-cultures, small communities and more complex communities). This valuable knowledge can only be acquired using pure culture, a strategy that is recommended to design future biotherapeutics as it allows a careful selection and screening of the species present in the community.

In contrast, top-down approaches identify molecular interaction networks based on correlated molecular behaviour obtained from complex communities. They allow naturally assembled communities to grow and therefore should be more resilient to perturbations. In this thesis, the top-down approach added crucial information about interactions in communities exposed to different substrate types. The enriched communities showed to be highly different, e.g., as a result of differences in the initial community structure at the onset of the enrichment process. We observed that polysaccharides led to more diverse communities, while intermediate metabolites (e.g., lactate) or small molecules (e.g., glucose) led to simpler communities. From the practical point of view, the resulting communities are less predictable and would be more difficult to standardise for use as biotherapeutics.

Likely, a combination of both approaches may be necessary for a comprehensive understanding of the structure and function of microbial communities and their role in human health and disease. Each approach has its strengths and limitations, and the use of simplified communities can provide novel insights into the coexistence of different bacterial strains within the gut environment. This thesis employed both approaches, initially constructing a framework to design defined communities by developing a culture collection and testing the defined community resilience in vitro. Simultaneously, the top-down approach provided greater insight into complex community compositions and interactions by utilising a bottleneck, the substrate added to repeated batch cultivation (Figure 5, left). Although both approaches are complementary, they could be used more iteratively. A future implementation of that is shown in Figure 5 (right panel), where the top-down approach will increase our knowledge on species interactions in complex communities while allowing enrichment of specific taxa in order to facilitate isolation efforts. These isolated strains could then be used to build a well-defined community from the bottom-up to target a specific community composition or function based on the knowledge acquired

in the top-down communities as well as the strain-specific in-depth metabolic characterisation.

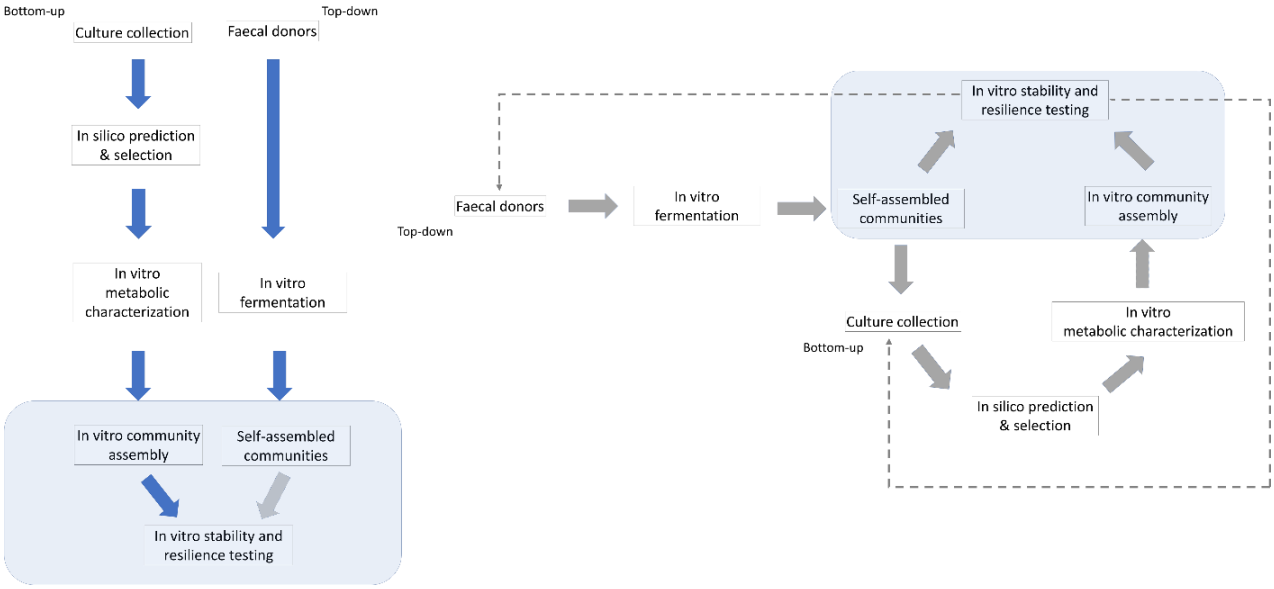


Figure 5. The left panel represents the current approach used in this thesis where both bottom-up and top-down are used in parallel to study communities (the grey arrow represents an additional way to study the top-down communities that was not investigated in this study). The right panel represents a possible future approach using both methods to better design next-generation therapeutics with possible feedback loops between the top-down and bottom-up approaches (dashed arrows). The blue box shows where community interactions can be investigated.

Studying gut communities: what comes next

Like most studies in the microbiome field, this thesis faced a number of limitations. One of the most apparent limitations is the use of simplified communities to investigate the gut environment, as the findings obtained in such conditions can be challenging to extrapolate to the entire ecosystem. The gut is composed of numerous different species, and studying only a subset, whether through the bottom-up approach (10 species) or the top-down approach (up to ~60 species), is still not entirely representative of the interactions that occur in the gut. On the other hand, given the high complexity of this ecosystem, only by breaking it down into simpler models can we begin to comprehend the role of each species in a complex setting. In this thesis, the use of different levels of complexity, as well as the parallel in silico and in vitro approaches to investigate competition, cooperation, and metabolic potential, demonstrated that more knowledge can be gained by employing these

methods. Moreover, other -omics methods could also be added to the methods used in this thesis, such as transcriptomics[151] to further elucidate with species is performing which function. For the top-down approach the use of more donors on several selected media including all the different enterotypes (including Bact2 and Rum) could also bring more insight on the different community compositions derived from specific substrates.

Another limitation of this thesis is the lack of host components in our in vitro system. Our work mainly focused on batch culture methods (Figure 6 top left), and while these methods allow high parallelization and high throughput[117], they are closed systems. Other methods such as continuous cultures using a chemostat or the SHIME, allow in and outflow of medium maintaining bacterial growth rate and physiological state (Figure 6 top right). In order to make these in vitro models physiologically more relevant, more complex fermentation configurations have been developed to mimic colonic systems. These models mimic the kinetic characteristics of the major compartments of the intestinal tract[110] and have been used to study the impact of certain substrates such as inulin[111] as well as to investigate the effect of plant-derived compounds on the gut-like ecosystem[112]. More advanced in vitro colon simulators add another layer of complexity to in vitro validation of the consortium by also including host parameters such as mucus layer and human cell lines[113] (Figure 6 bottom). The incorporation of mammalian cell components such as intestinal organoids, 2D cell models and transwell cultures allows the study of host responses of colonocytes and immune cells to microbial consortia and their metabolic outputs[114–116]. The host-microbe interactions envisaged here range from immune responses, exchange of metabolites to adhesion properties of bacterial cells.

In vitro simulations not only allow investigating intrinsic features of synthetic gut ecosystems but can also simulate effects imposed by chemical or biological perturbations. For instance, the stability of the synthetic community can be challenged by introduction of biological perturbations (e.g., *Clostridioides difficile*) and subsequent evaluation of its pathogen displacement potential. A further level of complexity can be achieved by the use of the above described colonic model systems to test interactions between designed consortia and the dysbiotic microbiota of patients from which the restoration potential of bacterial consortium can be explored.

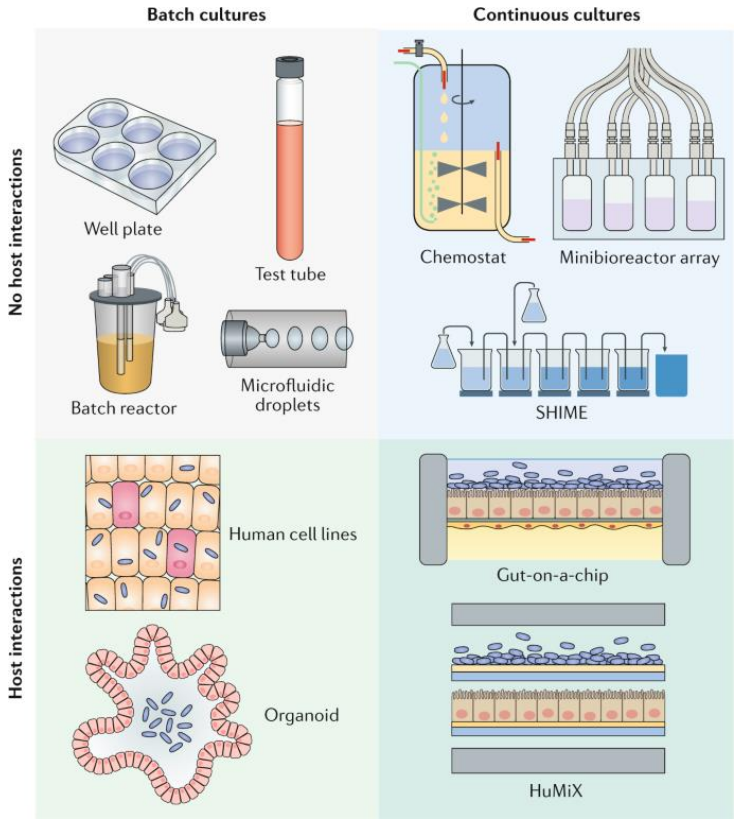


Figure 6. The current in vitro systems are either batch cultures, without or with host interactions, or continuous cultures, also without or with host interactions. At the top, the common system without host interactions while at the bottom, system designed to simulate gut human–microbiota interactions, figure from[117].

Despite the advances made in vitro, it is essential to investigate in vivo models to gain a better understanding of community interactions and the potential of consortia to restore an imbalanced ecosystem. However, even in vivo studies in humans have their limitations, as analyses are mostly performed on stool samples, which do not fully represent the complexity of the gut ecosystem. Cohort or clinical trial studies can provide valuable insights into the effect of dietary changes, drug-microbiota interactions, and the association between bacterial composition and a disease state. Nonetheless, true insights into the real spatial dynamics of the ecosystem are still lacking. Recent studies have highlighted the importance of spatial distribution in the gut and its functional consequences on community dynamics[213–215]. In vivo measurements using probes such as used in the recent study of De la Paz et al.[216] could be used to monitor glucose dynamics in real-time and open new avenues of metabolite monitoring. Future work on the capsule sensing platform aims to

overcome its current limitations and expand its functionality as well as integrating functionalities such as tracking oxygen concentration, temperature, and pH.

Other probes could be used to sample specific parts of the colon to allow a more representative view of the gut microbiota composition in situ[217]. Therefore, in addition to in vitro studies as described in this thesis, new technologies will allow the field to better study interactions. Adding complexity and looking at the system from different perspectives, from molecules to communities, is necessary to unravel the full host-microbiota interactions. It is also important to study not only the bacterial component of the microbiota but all organisms, including viruses, and eukaryotes to gain a complete understanding of this community. At each step, studying simple communities will still be valuable to better characterise microbe-to-microbe interactions and characterise communities for next-generation probiotics.

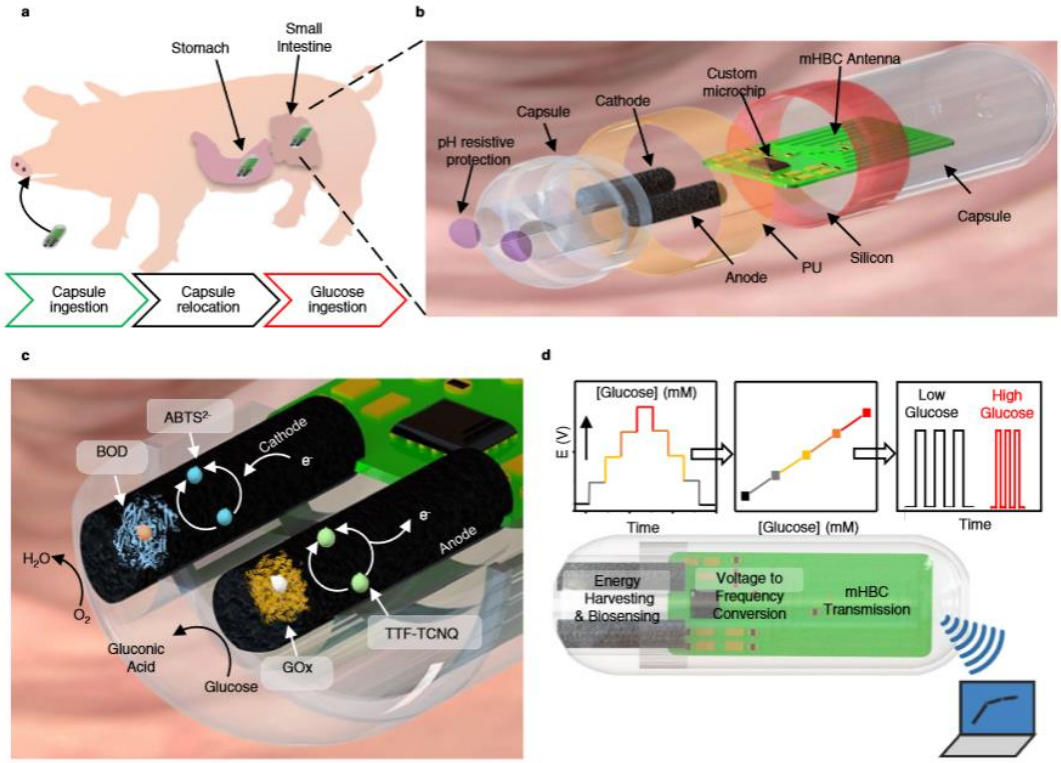


Figure 7. Probe for glucose measurements in vivo. (a) Schematics of the capsule sensor operating in a porcine model. (b) Schematic layout of the capsule sensor. (c) Illustration of the chemical composition and reaction mechanisms inside the sensor. (d) Signal conversion mechanism converting glucose concentrations to voltage, transmitted wirelessly to an external receiver adapted from [216].

Conclusion: from isolates to next-generation probiotics

Novel multistrain anaerobic communities reconstituted from gut isolates, with in vitro proven stability and production of anti-inflammatory metabolites such as butyric acid, are a promising approach for patients with microbiota-associated disorders. The portfolio of approaches to design such communities offers the opportunity to systematically develop and test specific bacterial cocktails to restore homeostasis in a health-promoting configuration. Following up on in vitro evaluations to ensure the safety of the strains themselves, further work is necessary to validate synthetic multistrain communities in in vivo models to study their colonization potential, safety, tolerance and restoration effects on disease models. Finally, safety studies in animals and intervention studies in humans are the ultimate hurdles before applicability and effectiveness of the synthetic consortium to modulate dysbiotic microbiota associated with the targeted disorder can be fully validated. This thesis addressed many challenges necessary to design next-generation probiotics including strain selection as well as the design of the community by studying its interactions and resilience, more challenges remain. Still challenges remain in order to develop effective formulations and delivery systems for probiotics to ensure their efficacy. Next-generation probiotics will need to be designed with improved delivery systems, such as encapsulation technologies or advanced coatings, to ensure that they reach their intended destination. Upscaling the production of such communities can also be challenging to produce large quantities of high-quality probiotic considering the need for anaerobic conditions and therefore will need to be designed with scalability in mind to meet the increasing demand.

Supplementary material

Supplementary material chapter 2

Supplementary Table S2.1 Gradients in physicochemical and biological parameters of the 11 donor samples.

Parameter	Range
pH	5.58 - 8.15
Water activity	0.973 - 1.000
Frozen cell count	$4.21x10^{10}$ - $4.04x10^{11}$ cells/g
Moisture content	49.8 - 81.9 %

Supplementary Table S2.2. Cell counts of the cultured fractions for each individual and each preservation condition.

Sample_ID	Preservation	Dilution	Replicate	Cultured_fraction_cell_count
SC03	P1	D-6	1	5.36E+09
SC03	P1	D-6	2	5.42E+09
SC03	P1	D-6	3	5.62E+09
SC03	P1	D-7	1	7.40E+08
SC03	P1	D-7	2	6.00E+08
SC03	P1	D-7	3	8.20E+08
SC03	P2	D-5	1	5.28E+09
SC03	P2	D-5	2	4.88E+09
SC03	P2	D-5	3	5.06E+09
SC03	P2	D-6	1	1.34E+09
SC03	P2	D-6	2	1.20E+09
SC03	P2	D-6	3	1.04E+09
SC03	P3	D-6	1	1.72E+09
SC03	P3	D-6	2	1.76E+09
SC03	P3	D-6	3	1.62E+09
SC03	P4	D-6	1	3.80E+09
SC03	P4	D-6	2	3.20E+09
SC03	P4	D-6	3	2.92E+09
SC03	P4	D-7	1	4.80E+09
SC03	P4	D-7	2	4.60E+09
SC03	P4	D-7	3	6.00E+09
SC18	P1	D-6	1	9.20E+08
SC18	P1	D-6	2	9.80E+08
SC18	P1	D-6	3	9.20E+08
SC18	P2	D-5	1	4.02E+09
SC18	P2	D-5	2	3.40E+09
SC18	P2	D-5	3	3.54E+09
SC18	P2	D-6	1	8.60E+08
SC18	P2	D-6	2	7.20E+08
SC18	P2	D-6	3	7.80E+08
SC18	P3	D-6	1	1.90E+09
SC18	P3	D-6	2	1.80E+09

SC18	P3	D-6	3	1.88E+09
SC18	P3	D-7	1	3.20E+09
SC18	P3	D-7	2	2.00E+09
SC18	P3	D-7	3	3.20E+09
SC18	P4	D-6	1	2.46E+09
SC18	P4	D-6	2	2.02E+09
SC18	P4	D-6	3	3.08E+09
SC22	P1	D-6	1	1.38E+09
SC22	P1	D-6	2	1.54E+09
SC22	P1	D-6	3	1.26E+09
SC22	P2	D-5	1	2.66E+09
SC22	P2	D-5	2	2.38E+09
SC22	P2	D-5	3	2.28E+09
SC22	P2	D-6	1	4.40E+08
SC22	P2	D-6	2	2.80E+08
SC22	P2	D-6	3	5.20E+08
SC22	P3	D-6	1	7.60E+08
SC22	P3	D-6	2	8.60E+08
SC22	P3	D-6	3	8.80E+08
SC22	P4	D-6	1	8.00E+08
SC22	P4	D-6	2	1.04E+09
SC22	P4	D-6	3	9.00E+08
SC08	P1	D-6	1	1.40E+09
SC08	P1	D-6	2	2.00E+09
SC08	P1	D-6	3	1.42E+09
SC08	P2	D-6	1	3.24E+09
SC08	P2	D-6	2	4.02E+09
SC08	P2	D-6	3	3.72E+09
SC08	P2	D-7	1	5.40E+09
SC08	P2	D-7	2	2.80E+09
SC08	P2	D-7	3	6.40E+09
SC08	P3	D-6	1	3.64E+09
SC08	P3	D-6	2	3.62E+09
SC08	P3	D-6	3	3.06E+09
SC08	P3	D-7	1	5.60E+09
SC08	P3	D-7	2	2.40E+09
SC08	P3	D-7	3	4.00E+09
SC08	P4	D-6	1	3.88E+09
SC08	P4	D-6	2	4.24E+09
SC08	P4	D-6	3	3.98E+09
SC08	P4	D-7	1	3.80E+09
SC08	P4	D-7	2	3.40E+09
SC08	P4	D-7	3	3.80E+09
SC36	P1	D-6	1	1.46E+09
SC36	P1	D-6	2	1.80E+09
SC36	P1	D-6	3	1.64E+09

SC36	P1	D-7	1	2.40E+08
SC36	P1	D-7	2	1.80E+08
SC36	P1	D-7	3	1.40E+08
SC36	P2	D-5	1	4.98E+09
SC36	P2	D-5	2	5.72E+09
SC36	P2	D-5	3	4.64E+09
SC36	P2	D-6	1	9.60E+08
SC36	P2	D-6	2	9.20E+08
SC36	P2	D-6	3	8.60E+08
SC36	P3	D-5	1	2.94E+07
SC36	P3	D-5	2	3.18E+07
SC36	P3	D-5	3	2.78E+07
SC36	P3	D-6	1	1.00E+09
SC36	P3	D-6	2	1.16E+09
SC36	P3	D-6	3	1.20E+09
SC36	P4	D-5	1	3.26E+07
SC36	P4	D-5	2	2.90E+07
SC36	P4	D-5	3	3.20E+07
SC36	P4	D-6	1	1.36E+09
SC36	P4	D-6	2	1.34E+09
SC36	P4	D-6	3	1.40E+09
SC21	P1	D-5	1	3.78E+08
SC21	P1	D-5	2	3.30E+08
SC21	P1	D-5	3	2.80E+08
SC21	P1	D-6	1	5.60E+08
SC21	P1	D-6	2	8.00E+08
SC21	P1	D-6	3	1.28E+09
SC21	P2	D-6	1	2.82E+09
SC21	P2	D-6	2	3.26E+09
SC21	P2	D-6	3	3.30E+09
SC21	P2	D-7	1	6.00E+09
SC21	P2	D-7	2	7.40E+09
SC21	P2	D-7	3	4.20E+09
SC21	P3	D-6	1	4.86E+09
SC21	P3	D-6	2	4.88E+09
SC21	P3	D-6	3	4.22E+09
SC21	P3	D-7	1	4.00E+09
SC21	P3	D-7	2	2.00E+09
SC21	P3	D-7	3	3.40E+09
SC21	P4	D-6	1	3.40E+09
SC21	P4	D-6	2	3.58E+09
SC21	P4	D-6	3	3.80E+09
SC21	P4	D-7	1	7.00E+09
SC21	P4	D-7	2	5.60E+09
SC21	P4	D-7	3	4.00E+09
SC17	P1	D-6	1	1.34E+09

SC17	P1	D-6	2	1.52E+09
SC17	P1	D-6	3	1.24E+09
SC17	P2	D-6	1	2.38E+09
SC17	P2	D-6	2	1.96E+09
SC17	P2	D-6	3	2.28E+09
SC17	P2	D-7	1	5.20E+09
SC17	P2	D-7	2	3.60E+09
SC17	P2	D-7	3	3.20E+09
SC17	P3	D-6	1	5.60E+08
SC17	P3	D-6	2	6.80E+08
SC17	P3	D-6	3	6.60E+08
SC17	P4	D-6	1	1.34E+09
SC17	P4	D-6	2	1.82E+09
SC17	P4	D-6	3	1.68E+09
SC17	P4	D-7	1	4.00E+09
SC17	P4	D-7	2	4.20E+09
SC17	P4	D-7	3	2.00E+09
SC23	P1	D-6	1	1.60E+09
SC23	P1	D-6	2	2.16E+09
SC23	P1	D-6	3	1.70E+09
SC23	P2	D-6	1	3.68E+09
SC23	P2	D-6	2	4.44E+09
SC23	P2	D-6	3	3.94E+09
SC23	P2	D-7	1	4.80E+09
SC23	P2	D-7	2	4.20E+09
SC23	P2	D-7	3	4.60E+09
SC23	P3	D-7	1	1.22E+10
SC23	P3	D-7	2	1.04E+10
SC23	P3	D-7	3	1.08E+10
SC23	P4	D-7	1	8.60E+09
SC23	P4	D-7	2	1.18E+10
SC41	P1	D-7	1	7.80E+08
SC41	P1	D-7	2	7.20E+08
SC41	P1	D-7	3	7.60E+08
SC41	P2	D-7	1	7.40E+09
SC41	P2	D-7	2	9.20E+09
SC41	P2	D-7	3	9.00E+09
SC41	P3	D-7	1	1.06E+10
SC41	P3	D-7	2	9.00E+09
SC41	P3	D-7	3	1.00E+10
SC41	P4	D-7	1	1.90E+10
SC41	P4	D-7	2	1.66E+10
SC41	P4	D-7	3	1.40E+10
SC42	P1	D-5	1	3.80E+07
SC42	P1	D-5	2	2.60E+07
SC42	P1	D-5	3	3.40E+07

SC42	P2	D-4	1	1.78E+07
SC42	P2	D-4	2	3.52E+07
SC42	P2	D-4	3	1.44E+07
SC42	P3	D-4	1	1.14E+07
SC42	P3	D-4	2	1.12E+07
SC42	P3	D-4	3	1.36E+07
SC42	P4	D-4	1	1.28E+07
SC42	P4	D-4	2	1.44E+07
SC42	P4	D-4	3	1.28E+07
SC29	P1	D-5	1	3.98E+08
SC29	P1	D-5	2	3.12E+08
SC29	P1	D-5	3	4.16E+08
SC29	P1	D-6	1	4.60E+08
SC29	P1	D-6	2	5.00E+08
SC29	P1	D-6	3	3.60E+08
SC29	P2	D-5	1	4.78E+09
SC29	P2	D-5	2	5.96E+09
SC29	P2	D-5	3	6.46E+09
SC29	P2	D-6	1	1.58E+09
SC29	P2	D-6	2	5.00E+08
SC29	P2	D-6	3	8.80E+08
SC29	P3	D-6	1	3.28E+09
SC29	P3	D-6	2	3.00E+09
SC29	P3	D-6	3	3.38E+09
SC29	P3	D-7	1	3.40E+09
SC29	P3	D-7	2	3.20E+09
SC29	P3	D-7	3	2.80E+09
SC29	P4	D-6	1	4.10E+09
SC29	P4	D-6	2	3.82E+09
SC29	P4	D-6	3	3.86E+09
SC29	P4	D-7	1	9.80E+09
SC29	P4	D-7	2	8.60E+09
SC29	P4	D-7	3	8.80E+09

Supplementary Table S2.3. Cumulative and independent contribution of metadata variables to community variation of the cultured fractions (dbRDA and stepwise dbRDA; FDR by Benjamini-Hochberg) in the cohort (n=129). Cumulative explanatory power and significance level of the included variables are reported.

dbRDA assuming variable independence

Metadata	Fmodel	R2	p-value	BH adj p-value
Preservation	1.03953	0.027328	0.407592	0.407592
Dilution	1.889848	0.048595	0.015984	0.018648
Frozen.cell.count.average	18.44472	0.581949	0.000999	0.001399
moisture_content	18.33503	0.139605	0.000999	0.001399
pH.average	7.676318	0.063611	0.000999	0.001399
water.activity.average	14.57956	0.114278	0.000999	0.001399
Individual	22.20622	0.655575	0.000999	0.001399

stepwise dbRDA

Metadata	R2.adj	Df	AIC	F	Pr..F.
Individual	0.63291	9	339.5874	1.78E+01	1.00E-04
Dilution	0.6579	3	336.7598	2.71E+00	1.00E-04

Supplementary Table S2.4: Bray-curtis dissimilarity distances for each sample (n=129) to its respective feacal sample.

Sample	Bray-Curtis distance to faecal sample
SC03_P1_10.4	0.930327869
SC03_P1_10.5	0.927595628
SC03_P1_10.6	0.949453552
SC03_P2_10.4	0.926229508
SC03_P2_10.5	0.930327869
SC03_P2_10.6	0.93579235
SC03_P3_10.4	0.943989071
SC03_P3_10.5	0.952185792
SC03_P3_10.6	0.941256831
SC03_P4_10.4	0.942622951
SC03_P4_10.5	0.93715847
SC03_P4_10.6	0.959016393
SC08_P1_10.4	0.961748634
SC08_P1_10.5	0.963114754
SC08_P1_10.6	0.931693989
SC08_P2_10.4	0.93442623
SC08_P2_10.5	0.922131148
SC08_P2_10.6	0.93852459
SC08_P3_10.4	0.908469945
SC08_P3_10.5	0.909836066
SC08_P3_10.6	0.908469945

SC08_P4_10.4	0.908469945
SC08_P4_10.5	0.908469945
SC08_P4_10.6	0.908469945
SC17_P1_10.4	0.983606557
SC17_P1_10.5	0.956284153
SC17_P1_10.6	0.855191257
SC17_P2_10.4	0.975409836
SC17_P2_10.5	0.953551913
SC17_P2_10.6	0.953551913
SC17_P3_10.3	0.960382514
SC17_P3_10.4	0.946721311
SC17_P3_10.5	0.819672131
SC17_P4_10.4	0.965846995
SC17_P4_10.5	0.821038251
SC17_P4_10.6	0.819672131
SC18_P1_10.3	0.781420765
SC18_P1_10.4	0.781420765
SC18_P1_10.5	0.786885246
SC18_P2_10.3	0.771857923
SC18_P2_10.4	0.782786885
SC18_P2_10.5	0.782786885
SC18_P3_10.4	0.785519126
SC18_P3_10.5	0.786885246
SC18_P3_10.6	0.789617486
SC18_P4_10.4	0.785519126
SC18_P4_10.5	0.777322404
SC18_P4_10.6	0.788251366
SC21_P1_10.3	0.745901639
SC21_P1_10.4	0.781420765
SC21_P1_10.5	0.762295082
SC21_P2_10.4	0.743169399
SC21_P2_10.5	0.75136612
SC21_P2_10.6	0.773224044
SC21_P3_10.4	0.819672131
SC21_P3_10.5	0.784153005
SC21_P3_10.6	0.767759563
SC21_P4_10.4	0.740437158
SC21_P4_10.5	0.760928962
SC21_P4_10.6	0.75273224
SC22_P1_10.3	0.841530055
SC22_P1_10.4	0.81557377
SC22_P1_10.5	0.800546448
SC22_P2_10.3	0.834699454
SC22_P2_10.4	0.801912568
SC22_P2_10.5	0.841530055
SC22_P3_10.3	0.844262295

SC22_P3_10.4	0.837431694
SC22_P3_10.5	0.893442623
SC22_P4_10.3	0.834699454
SC22_P4_10.4	0.838797814
SC22_P4_10.5	0.897540984
SC23_P1_10.4	0.919398907
SC23_P1_10.5	0.769125683
SC23_P1_10.6	0.628415301
SC23_P2_10.4	0.946721311
SC23_P2_10.5	0.740437158
SC23_P2_10.6	0.612021858
SC23_P3_10.4	0.952185792
SC23_P3_10.5	0.607923497
SC23_P3_10.6	0.754098361
SC23_P4_10.4	0.871584699
SC23_P4_10.5	0.81147541
SC23_P4_10.6	0.607923497
SC29_P1_10.3	0.923497268
SC29_P1_10.4	0.912568306
SC29_P1_10.5	0.922131148
SC29_P2_10.3	0.924863388
SC29_P2_10.4	0.928961749
SC29_P2_10.5	0.928961749
SC29_P3_10.4	0.911202186
SC29_P3_10.5	0.920765027
SC29_P3_10.6	0.923497268
SC29_P4_10.4	0.915300546
SC29_P4_10.5	0.922131148
SC29_P4_10.6	0.972677596
SC36_P1_10.4	0.823770492
SC36_P1_10.5	0.81557377
SC36_P1_10.6	0.819672131
SC36_P2_10.4	0.823770492
SC36_P2_10.5	0.825136612
SC36_P2_10.6	0.826502732
SC36_P3_10.4	0.826502732
SC36_P3_10.5	0.849726776
SC36_P3_10.6	0.81420765
SC36_P4_10.4	0.836065574
SC36_P4_10.5	0.846994536
SC36_P4_10.6	0.844262295
SC41_P1_10.4	0.924863388
SC41_P1_10.5	0.922131148
SC41_P1_10.6	0.81147541
SC41_P2_10.4	0.931693989
SC41_P2_10.5	0.870218579

SC41_P2_10.6	0.864754098
SC41_P3_10.4	0.905737705
SC41_P3_10.5	0.885245902
SC41_P3_10.6	0.801912568
SC41_P4_10.4	0.786885246
SC41_P4_10.5	0.785519126
SC41_P4_10.6	0.785519126
SC42_P1_10.3	0.978142077
SC42_P1_10.4	0.965846995
SC42_P1_10.5	0.825136612
SC42_P2_10.3	0.979508197
SC42_P2_10.4	0.856557377
SC42_P3_10.3	0.825136612
SC42_P3_10.4	0.825136612
SC42_P4_10.3	0.816939891
SC42_P4_10.4	0.825136612

Supplementary Table S2.5. Relative abundances of genera detected in faecal and cultured fractions samples in culture-independent (faecal) and cultured fractions per preservation conditions (P1 to P4).

Genus	Faecal	P1	P2	P3	P4	Average (P1-P4)
Faecalibacterium	0.187206	0	0	0	0	0
Phocaeicola	0.107986	0.092641	0.143843	0.16993	0.248975	0.163847366
Bacteroides	0.07046	0.203044	0.21519	0.286128	0.30372	0.252020566
Agathobacter	0.053805	0	0	0	0	0
Blautia_A	0.049818	0	0	0	0	0
Roseburia	0.03307	0	0	0	0	0
Gemmiger	0.031871	0	0	0	0	0
Bifidobacterium	0.028796	0.48798	0.508188	0.333096	0.264959	0.398555798
Alistipes	0.02644	0	0	0	0	0
Prevotella	0.02608	0	0	0	0	0
Ruminococcus_E	0.021203	0	0	0	0	0
Erysipelatoclostridium	0.021128	0	0	0.000331	0.00061	0.00023541
Dorea	0.015884	0.000152	0.000103	0	0.00029	0.00013624
Anaerostipes	0.013717	0	0	0	0	0
Fusicatenibacter	0.013381	0	0	0	0	0
Lachnospira	0.012774	0	0	0	0	0
Acetatifactor	0.012604	0	0	0	0	0
Anaerobutyricum	0.012115	0	0	0	0	0
Coprococcus	0.010794	0	0	0	0	0
CAG-831	0.009709	0.001289	0.002114	0.004004	0.002408	0.002453666
Prevotellamassilia	0.008309	0.044709	0.04831	0.035389	0.030811	0.039804844
Mediterraneibacter	0.007576	0	0.000106	0	0	2.65751E-05
uc_f_Lachnospiraceae	0.006905	0	0	0	0	0

Parabacteroides	0.00677	0.016393	0.016792	0.031922	0.033566	0.024668402
Dialister	0.006448	0.000318	0.000325	0.001047	0.002648	0.001084682
UBA11471	0.006308	0	0	0	0	0
ER4	0.006055	0	0	0	0	0
UMGS1994	0.006032	0	0	0	0	0
Bariatricus	0.00586	0	0	0	0	0
Enterococcus_B	0	0.003734	0.003711	0.004111	0.002098	
Methanobrevibacter_A	0.005791	0	0	0	0	0
Ruminococcus_D	0.005269	0	0	0	0	0
Collinsella	0.005177	0.023754	0.007363	0.00155	0.003318	0.008996412
CAG-83	0.004794	0	0	0	0	0
UBA7173	0.004688	0	0	0	0	0
Agathobaculum	0.004545	0	0	0	0	0
Akkermansia	0.004391	0	0	0	0	0
Streptococcus	0.004371	0.00027	0.000766	0.000281	0.001667	0.000746178
Eubacterium_R	0.004283	0	0	0	0	0
Phascolarctobacterium	0.004051	0.00384	0.002395	0.002935	0.006446	0.003904065
CAG-110	0.003766	0	0	0	0	0
Eubacterium_G	0.003477	0	0	0	0	0
TF01-11	0.003054	0	0	0	0	0
Sutterella	0.003022	0.000394	0	0	0.000227	0.000155264
Eubacterium_F	0.002877	0	0	0	0	0
Enterocloster	0.002861	0.0001	0	0	0	2.50218E-05
Acutalibacter	0.002856	0	0	0	0	0
uc_f_Bacteroidaceae	0.002845	0	0	0	0	0
Catenibacterium	0.002748	0	0	0	0	0
Butyrivibrio_A	0.002696	0	0	0	0	0
Eisenbergiella	0.002696	0	0	0	0	0
RUG714	0.002659	0	0	0	0	0
UMGS1375	0.002633	0	0	0	0	0
Ruminococcus_A	0.00261	0	0	0	0	0
Succinivibrio	0.002567	0	0	0.000272	0	6.79896E-05
uc_f_Eggerthellaceae	0.002565	0	0	0	0	0
Clostridium_Q	0.002259	0.000352	0	0	0	8.79555E-05
Coprococcus_A	0.002243	0	0	0	0	0
CAG-873	0.002215	0	0	0	0	0
CAG-41	0.002169	0	0	0	0	0
UBA7182	0.0021	0	0	0	0	0
CAG-81	0.001949	0	0	0	0	0
Oscillibacter	0.001946	0	0	0	0	0
uc_f_CAG-508	0.001889	0	0	0	0	0
CAG-45	0.001845	0	0	0	0	0
CAG-353	0.001769	0	0	0	0	0
Bilophila	0.001721	0	0	0	0	0
CHKCI001	0.001622	0	0	0	0	0
Eubacterium_I	0.001427	0	0	0	0	0

CAG-462	0.001386	0.000158	0.000485	0.000116	0.000303	0.000265364
Tidjanibacter	0.001372	0	0	0	0	0
Parasutterella	0.001328	0	0	0.000144	0	3.59485E-05
Holdemanella	0.001319	0	0	0	0	0
Adlercreutzia	0.001287	0	0	0	0	0
CAG-488	0.001262	0	0	0	0	0
Anaerotignum	0.001227	0	0	0	0	0
UBA1191	0.001209	0	0	0	0	0
Enterococcus_A	0	0.003649	0.00031	0.001588	0.001237	
CAG-1427	0.001204	0	0	0	0	0
uc_c_Clostridia	0.001181	0	0	0	0	0
SFTJ01	0.001172	0	0	0	0	0
CAG-279	0.00117	0	0	0	0	0
OEMS01	0.001128	0	0	0	0	0
Ruminococcus_C	0.001089	0	0	0	0	0
Alistipes_A	0.001069	0	0	0	0	0
Flavonifractor	0.001048	0.000989	0.000331	0.000494	0.00057	0.00059608
Mailhella	0.001013	0	0	0	0	0
uc_o_Oscillospirales	0.00097	0	0	0	0	0
Ruminiclostridium_E	0.000967	0	0	0	0	0
Odoribacter	0.000938	0.000112	0.002683	0.001691	0.003568	0.00201356
PeH17	0.000935	0	0	0	0	0
uc_f_Acutalibacteraceae	0.000933	0	0	0	0	0
CAG-603	0.000889	0	0	0	0	0
51-20	0.000887	0	0	0	0	0
Zag111	0.000885	0	0	0	0	0
Romboutsia	0.000862	0	0	0	0	0
UBA3792	0.000859	0	0	0	0	0
CAG-127	0.000857	0	0	0	0	0
Clostridium_N	0.000853	0	0	0	0	0
Staphylococcus	0	0.00202	0	0	0	
Alloprevotella	0	0	0	0	0	
Blautia	0.000827	0	0	0	0	0
GCA-900066135	0.000825	0	0	0	0	0
Clostridium_P	0	0.001586	0.000444	0	0.000564	
Intestinimonas	0.000825	0	0	0	0.000137	3.41828E-05
Senegalimassilia	0.000797	0	0	0	0	0
Terrisporobacter	0	0	0	0	0	
Lawsonibacter	0.000768	0	0	0	0	0
Eubacterium_C	0.000745	0	0	0	0	0
Enterobacter	0	0	0	0.001463	0.000567	
Clostridium	0.000731	0.001429	0	0	0	0.00035713
Peptoniphilus_A	0	0.00124	0.001282	0.000256	0.000987	
uc_p_Firmicutes_A	0.00071	0	0	0	0	0
Ruminococcus	0.000699	0	0	0	0	0
CAG-495	0.000648	0	0	0	0	0

UBA9502	0.000643	0	0	0	0	0
UMGS1071	0.000623	0	0	0	0	0
Paraprevotella	0.00062	0	0	0	0	0
Acidaminococcus	0.000597	0.017085	0.000535	0.011288	0.014967	0.010968538
Intestinibacter	0.000586	0	0	0	0	0
Veillonella	0.000584	0.002323	0	0.000103	0.000127	0.000638281
Evtepia	0.000581	0	0	0	0	0
uc_k_Bacteria	0.000552	0.001383	0.003789	0.003439	0.002591	0.002800526
Ruthenibacterium	0.000549	0	0	0	0	0
Citrobacter	0	0	0	0.001529	0	
Longibaculum	0.000533	0	0	0	0	0
Ruminococcus_B	0.000522	0	0	0	0	0
Firm-11	0.000512	0	0	0	0	0
CAG-465	0.000508	0	0	0	0	0
GCA-900066995	0.000473	0	0	0	0	0
Eggerthella	0.000471	0.001498	0	0.001797	0.001037	0.001083215
uc_f_Ruminococcaceae	0.000469	0	0	0	0	0
UBA2730	0.000439	0	0	0	0	0
Escherichia	0.000434	0.08515	0.039378	0.102719	0.069926	0.074293385
uc_f_Anaerovoracaceae	0.000414	0	0	0	0	0
Butyricicoccus	0.000395	0	0	0	0	0
Desulfovibrio	0.000384	0	0	0	0	0
Ligilactobacillus	0.000384	0	0.000209	0	0	5.23686E-05
RC9	0.000381	0	0	0	0	0
Coprobacter	0.000377	0	0	0.000606	0	0.000151609
GCA-900066575	0.000363	0	0	0	0	0
CAG-238	0.000336	0	0	0	0	0
CAG-475	0	0	0	0	0	
Longicatena	0.000336	0	0	0	0.00054	0.000135064
CAG-354	0.000333	0	0	0	0	0
QALR01	0.000315	0	0	0	0	0
Phascolarctobacterium_A	0.000306	0	0	0	0	0
UMGS1441	0.000301	0	0	0	0	0
CAG-302	0.000296	0	0	0	0	0
QAMM01	0.000287	0	0	0	0	0
Clostridium_A	0.00028	0	0	0	0	0
uc_f_Burkholderiaceae	0.000271	0	0	0.000184	0	4.61079E-05
CAG-196	0.000269	0	0	0	0	0
Hungatella_A	0.000262	0	0	0	0	0
Peptostreptococcus	0	0.000664	0	0	0	
Hungatella	0	0.000334	0	0.000228	0	
Negativibacillus	0.000257	0	0	0	0	0
UMGS1781	0.000234	0	0	0	0	0
UBA1820	0.00023	0	0	0	0	0
Schaedlerella	0.000223	0	0	0	0	0
Brachyspira	0.000223	0	0	0	0	0

CAG-484	0	0	0	0	0	
Kineothrix	0	0	0	0	0	
CAG-314	0	0	0	0	0	
uc_f_Enterobacteriaceae	0	0	0	0.000569	0	
OEMR01	0.000214	0	0	0	0	0
CAG-307	0.000207	0	0	0	0	0
Faecalicoccus	0	0	0	0	0	
uc_f_Muribaculaceae	0.000205	0	0	0	0	0
Turicibacter	0.000205	0.000376	0.000688	0	0.000303	0.000341847
Butyricimonas	0.000205	0	0	0	0	0
CAG-180	0	0	0	0	0	
Megasphaera	0.000198	0	0	0	0	0
Slackia_A	0.000188	0	0	0	0	0
Marvinbryantia	0.000179	0	0	0	0	0
UBA2883	0.000175	0	0	0	0	0
uc_o_Peptostreptococcales	0	0	0	0	0	
UBA7057	0.000172	0	0	0	0	0
OF09-33XD	0.00017	0	0	0	0	0
Lactobacillus	0	0.000185	0.000147	0	0.00012	
Oxalobacter	0.000154	0	0	0	0	0
Succiniclasticum	0.000145	0	0	0	0	0
Acetitomaculum	0	0	0	0	0	
uc_f_Oscillospiraceae	0.000142	0	0	0	0	0
G11	0	0	0	0	0	
Frisingicoccus	0.000142	0	0	0	0	0
uc_f_Erysipelatoclostridiaceae	0	0	0	0	0	
UBA6985	0.000136	0	0	0	0	0
CAG-115	0.000126	0	0	0	0	0
Emergencia	0.000126	0	0	0	0	0
Olsenella_E	0.000124	0	0	0	0	0
Mediterraneibacter_A	0	0	0	0	0	
Massiliomicrobiota	0.000115	0	0	0	0	0
AM51-8	0	0	0	0	0	
Mesosutterella	0	0	0	0	0	
Allisonella	0	0	0	0	0	
Finegoldia	0	0.000124	0	0	0	
Anaerotruncus	0	0	0	0	0	
Monoglobus	0.00011	0	0	0	0	0
CAG-313	0	0	0	0	0	
UBA9475	0	0	0	0	0	
Sporobacter	0.000106	0	0	0	0	0
Anaerococcus	0	0	0	0	0	
Ezakiella	0	0	0	0	0	
CAG-628	0	0	0	0	0	
uc_o_Lachnospirales	0	0	0	0	0	
Methanosphaera	0.000106	0	0	0	0	0

Parolsenella	0.000103	0	0	0	0	0
uc_f_Acetivibrionaceae	0	0	0	0	0	
UBA733	0	0	0	0	0	
Helicobacter_C	0	0	0	0	0	
Fenollaria	0	0	0.000163	0	0	
UBA6382	0	0	0	0	0	
Fournierella	0	0	0	0	0	
UMGS416	0	0	0	0	0	
CAG-95	0	0	0	0	0	
Peptoniphilus_C	0	0	0	0	0	
SFVR01	0	0	0	0	0	
Ruminococcus_F	0	0	0	0	0	
Anaeromassilibacillus	0	0	0	0	0	
uc_f_CAG-822	0.000101	0	0	0	0	0
uc_f_Gastranaerophilaceae	0	0	0	0	0	
CAG-878	0	0	0	0	0	
Ruminiclostridium	0	0	0	0	0	
Mitsuokella	0	0	0	0.000109	0	
UBA9414	0	0	0	0	0	
Eubacterium	0	0	0	0.0001	0	
Enteroscipio	0	0	0	0	0	
GCA-900066905	0	0	0	0	0	
Porphyromonas_A	0	0	0	0	0	
Anaerofustis	0	0	0	0	0	
Citrobacter_A	0	0	0	0	0	
uc_o_RF39	0	0	0	0	0	
Achromobacter	0	0	0	0	0	
Duncaniella	0	0	0	0	0	

Supplementary Table S2.6. Associations between richness in the cultured fractions and the original richness, the preservation conditions and dilutions (n=129).

	Estimate	glm.std.coef	glm.pvalue	glm.FDR
Dilution	0.0215882	0.0041725	9.03E-07	1.30E-06
PreservationP2	0.0144943	0.0109411	0.18771	
PreservationP3	0.0146594	0.0110342	0.18646	
PreservationP4	-0.0043553	0.0102989	0.67311	
RichnessF	-0.0008683	0.0002852	0.00285	0.003483

Supplementary Table S2.7. Summary of the number of isolates and the number of recovered sequences from 16S rDNA sequencing of the isolates per individual and preservation condition.

Individual	Preservation	Isolates	Sequenced	%
SC03	P1	94	82	0.87
SC03	P2	94	91	0.97
SC03	P3	94	85	0.90
SC03	P4	94	50	0.53
SC08	P1	94	57	0.61
SC08	P2	94	81	0.86
SC08	P3	94	69	0.73
SC08	P4	94	86	0.91
SC17	P1	94	39	0.41
SC17	P2	94	74	0.79
SC17	P3	72	69	0.73
SC17	P4	94	80	0.85
SC18	P1	94	84	0.89
SC18	P2	94	80	0.85
SC18	P3	94	86	0.91
SC18	P4	94	87	0.93
SC41	P1	94	86	0.91
SC41	P2	94	91	0.97
SC41	P3	94	92	0.98
SC41	P4	94	63	0.67
SC21	P1	94	87	0.93
SC21	P2	94	90	0.96
SC21	P3	94	88	0.94
SC21	P4	94	77	0.82
SC22	P1	94	80	0.85
SC22	P2	94	65	0.69
SC22	P3	89	89	0.95
SC22	P4	94	92	0.98
SC23	P1	94	63	0.67
SC23	P2	94	82	0.87
SC23	P3	94	94	1.00
SC23	P4	83	71	0.76
SC29	P1	94	77	0.82
SC29	P2	94	89	0.95
SC29	P3	94	69	0.73
SC29	P4	94	84	0.89
SC36	P1	94	45	0.48
SC36	P2	94	84	0.89
SC36	P3	94	85	0.90
SC36	P4	94	92	0.98
SC42	P1	94	48	0.51
SC42	P2	94	75	0.80
SC42	P3	84	70	0.74
SC42	P4	94	67	0.71
			Median	0.87

Supplementary Table S2.8. Proportion of taxa per total sequences for each preservation condition at genus and species level.

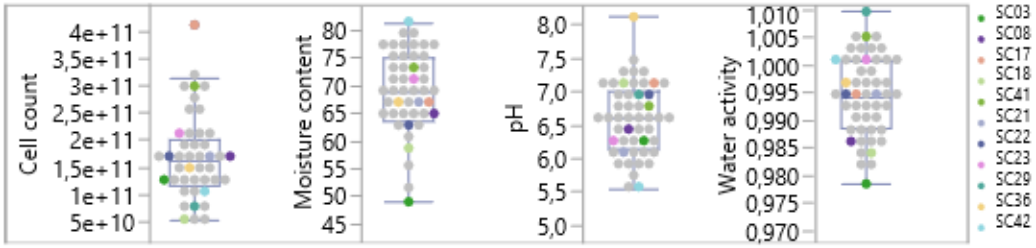
Preservation condition	Species	Genera	Total sequenced	Proportion of genera per total sequences	Proportion of species per total sequences	Proportion of genera in P1 compared to Px	Proportion of species in P1 compared to Px2
P1	72	36	686	5%	10%		
P2	62	30	804	4%	8%	1.41	1.36
P3	59	29	858	3%	7%	1.55	1.53
P4	66	33	774	4%	9%	1.23	1.23

Supplementary Table S2.9. Presence/absence of genera between culture-independent (feacal samples), cultured fractions and isolates, detection threshold >0.01%.

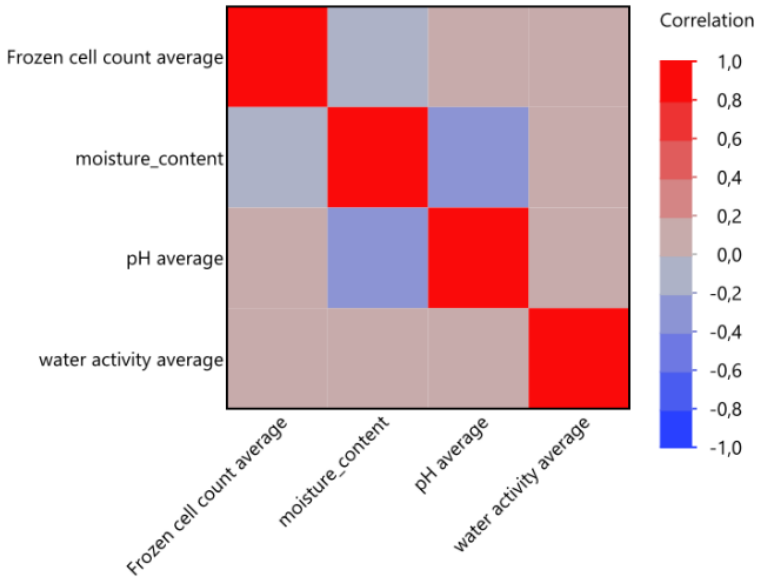
Genera	Isolated	Cultured fraction	Faecal samples
Acetatifactor			1
Acidaminococcus		1	
Acutalibacter			1
Adlercreutzia	1		1
Agathobacter			1
Agathobaculum			1
Akkermansia	1		1
Alistipes	1		1
Alistipes_A	1		1
Anaerobutyricum			1
Anaerostipes			1
Anaerotignum			1
Bacteroides	1	1	1
Bariatricus			1
Barnesiella	1		
Bifidobacterium	1	1	1
Bilophila	1		1
Blautia_A			1
Brachyspira			1
Butyricicoccus			1
Butyricimonas	1		
Butyrivibrio_A			1
CAG-462	1	1	1
CAG-831		1	1
Citrobacter		1	
Clostridium	1	1	
Clostridium_A	1		
Clostridium_N			1
Clostridium_P		1	

Clostridium_Q		1	1
Collinsella	1	1	1
Coprobacillus	1		
Coprobacter	1	1	
Coprococcus			1
Coprococcus_A			1
Cutibacterium	1		
Desulfovibrio	1		
Dialister		1	1
Dorea		1	1
Eggerthella	1	1	1
Eisenbergiella	1		1
Enorma	1		
Enterobacter		1	
Enterocloster	1	1	1
Enterococcus_A	1	1	
Enterococcus_B	1	1	
Enteroscipio	1		
Erysipelatoclostridium		1	1
Escherichia	1	1	1
Eubacterium	1	1	
Eubacterium_C			1
Eubacterium_F			1
Eubacterium_G			1
Eubacterium_I			1
Eubacterium_R			1
Evtepia			1
Faecalibacterium			1
Fenollaria		1	
Finegoldia		1	
Flavonifractor	1	1	1
Fusicatenibacter			1
Gemmiger			1
Gordonibacter	1		
Hafnia	1		
Holdemanella	1		1
Hungatella	1	1	
Intestinibacter			1
Intestinimonas	1	1	1
Lachnospira			1
Lactobacillus		1	
Lactococcus	1		
Lapidilactobacillus	1		
Lawsonibacter			1
Ligilactobacillus	1	1	

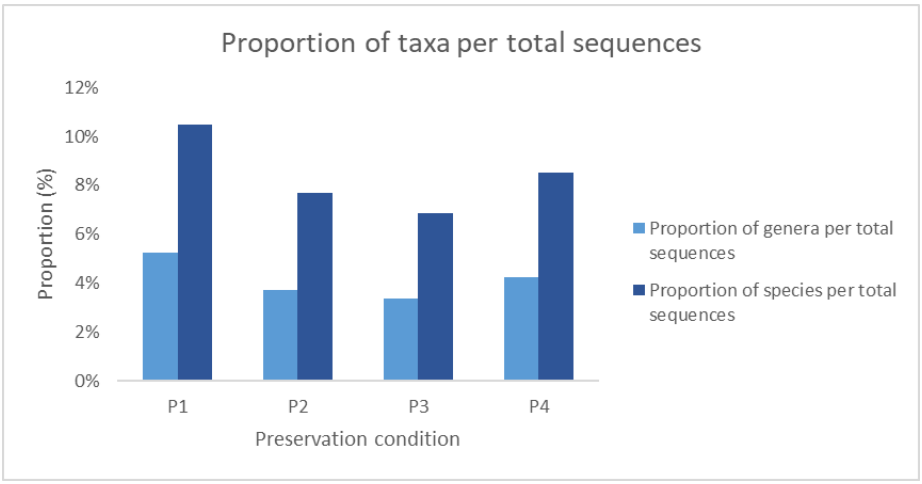
Longicatena	1	1	
Marseille-P4683	1		
Mediterraneibacter		1	1
Methanobrevibacter_A			1
Mitsuokella		1	
Odoribacter	1	1	1
Parabacteroides	1	1	1
Paraprevotella			1
Parasutterella		1	1
Parvimonas	1		
Pauljensenia	1		
Peptoniphilus_A	1	1	
Peptostreptococcus		1	
Phascolarctobacterium	1	1	1
Phocaeicola	1	1	1
Prevotella	1		1
Prevotellamassilia		1	1
Pseudomonas_E	1		
Raoultibacter	1		
Roseburia			1
Ruminiclostridium_E			1
Ruminococcus_A			1
Ruminococcus_B			1
Ruminococcus_C			1
Ruminococcus_D			1
Ruminococcus_E	1		1
Ruthenibacterium	1		1
Senegalimassilia			1
Staphylococcus	1	1	
Streptococcus	1	1	1
Succinivibrio		1	1
Sutterella		1	1
Tidjanibacter	1		
Turicibacter		1	
Varibaculum	1		
Veillonella	1	1	
Vibrio	1		



Supplementary Figure S2.1: Distribution of gradients among the 51 samples, selected samples are coloured.



Supplementary Figure S2.2: Spearman correlation heatmap of the faecal variables, no significance was observed.



Supplementary Figure S1.3: Proportion of taxa per total sequence per preservation conditions at species and genus level.

Supplementary material chapter 3

Supplementary Table S3.1 Detailed information about the strains retrieved from IMG

taxon_oid	Domain	Sequencing Status	Study Name	Genome Name / Sample Name	Sequencing Center	IMG Genome ID	Phylum	Family	NCBI Taxon ID	Strain
640612206	Bacteria	Permanent Draft	Human Microbiome Pr	Collinsella aerofaciens ATCC 25986	Washington University in St. Louis	640612206	Actinobacteria	Coriobacteriaceae	411903	ATCC 25986
2562617166	Bacteria	Permanent Draft	Human Microbiome Pr	Prevotella copri CB7, DSM 18205	Washington University in St. Louis	2562617166	Bacteroidetes	Prevotellaceae	537011	CB7, DSM 18205
650377925	Bacteria	Finished	Human Microbiome Pr	Coprococcus catus GD/7	Wellcome Trust Sanger Institute	650377925	Firmicutes	Lachnospiraceae	717962	GD/7
2811995281	Bacteria	Permanent Draft	Genome sequencing of	Parabacteroides merdae NCTC 13052	Wellcome Trust Sanger Institute	2811995281	Bacteroidetes	Tannerellaceae	46503	NCTC 13052
644736367	Bacteria	Finished	Eubacterium rectale AT	Eubacterium rectale ATCC 33656	Washington University in St. Louis	644736367	Firmicutes	Lachnospiraceae	515619	ATCC 33656
642979370	Bacteria	Permanent Draft	Human Microbiome Pr	Phocaeicola dorei DSM 17855	Washington University in St. Louis	642979370	Bacteroidetes	Bacteroidaceae	483217	DSM 17855
640963046	Bacteria	Permanent Draft	Human Microbiome Pr	Dorea longicatena DSM 13814	Washington University in St. Louis	640963046	Firmicutes	Lachnospiraceae	411462	DSM 13814
2756170247	Bacteria	Permanent Draft	Genomic Encyclopedia	Intestinimonas butyriciproducens DSM 26588	DOE Joint Genome Institute (JGI)	2756170247	Firmicutes	unclassified	1297617	DSM 26588
650377904	Bacteria	Permanent Draft	Human Microbiome Pr	Alistipes shahii WAL 8301	Wellcome Trust Sanger Institute	650377904	Bacteroidetes	Rikenellaceae	717959	WAL 8301, AY974072
640963024	Bacteria	Permanent Draft	Human Microbiome Pr	Blautia obeum ATCC 29174	Washington University in St. Louis	640963024	Firmicutes	Lachnospiraceae	411459	ATCC 29174
640753008	Bacteria	Finished	Bacteroides vulgatus AT	Bacteroides vulgatus ATCC 8482	Washington University in St. Louis	640753008	Bacteroidetes	Bacteroidaceae	435590	ATCC 8482
649633078	Bacteria	Finished	A Genomic Encyclopedi	Odoribacter splanchnicus 1651/6, DSM 20712	DOE Joint Genome Institute (JGI)	649633078	Bacteroidetes	Odoribacteraceae	709991	1651/6
2561511082	Bacteria	Permanent Draft	Genomic Encyclopedia	Agathobaculum desmolans ATCC 43058	DOE Joint Genome Institute (JGI)	2561511082	Firmicutes	Oscillospiraceae	1408437	ATCC 43058
2706794656	Bacteria	Permanent Draft	The genome sequence	Roseburia faecis M72	Wellcome Trust Sanger Institute	2706794656	Firmicutes	Lachnospiraceae	301302	M72
650377990	Bacteria	Finished	Human Microbiome Pr	Anaerostipes hadrus SSC/2	Wellcome Trust Sanger Institute	650377990	Firmicutes	Lachnospiraceae	649756	SSC/2
2871890851	Bacteria	Permanent Draft	Prevotella lascolaii KHD	Prevotella lascolaii KHD1	Research Unit on Emerging Infectious and Tropical Diseases (URMITE)	2871890851	Bacteroidetes	Prevotellaceae	1776379	KHD1
645951831	Bacteria	Permanent Draft	Human Microbiome Pr	Faecalibacterium prausnitzii A2-165	Washington University in St. Louis	645951831	Firmicutes	Oscillospiraceae	411483	A2-165
2693429854	Bacteria	Permanent Draft	Genomic Encyclopedia	Alistipes timonensis DSM 25383	DOE Joint Genome Institute (JGI)	2693429854	Bacteroidetes	Rikenellaceae	1033731	DSM 25383
2529292932	Bacteria	Permanent Draft	Human Microbiome Pr	Barnesiella intestinihominis YIT 11860	Broad Institute	2529292932	Bacteroidetes	Barnesiellaceae	742726	YIT 11860
643886116	Bacteria	Permanent Draft	Human Microbiome Pr	Coprococcus comes ATCC 27758	Washington University in St. Louis	643886116	Firmicutes	Lachnospiraceae	470146	ATCC 27758
2590828806	Bacteria	Permanent Draft	Genomic Encyclopedia	Gemmiger formicilis ATCC 27749	DOE Joint Genome Institute (JGI)	2590828806	Firmicutes	unclassified	745368	ATCC 27749
2693429911	Bacteria	Permanent Draft	Genomic Encyclopedia	Bacteroides uniformis DSM 6597	DOE Joint Genome Institute (JGI)	2693429911	Bacteroidetes	Bacteroidaceae	820	DSM 6597
641736133	Bacteria	Permanent Draft	Human Microbiome Pr	Dorea formicigenerans ATCC 27755	Washington University in St. Louis	641736133	Firmicutes	Lachnospiraceae	411461	ATCC 27755
2920298691	Bacteria	Permanent Draft	Sequencing of Lachnosp	Fusicatenibacter saccharivorans MSK.18.88	University of Chicago	2920298691	Firmicutes	Lachnospiraceae	1150298	MSK.18.88
2517572213	Bacteria	Permanent Draft	Genomic Encyclopedia	Alistipes onderdonkii WAL 8169, DSM 19147	DOE Joint Genome Institute (JGI)	2517572213	Bacteroidetes	Rikenellaceae	1203611	WAL 8169
2528768167	Bacteria	Permanent Draft	Genomic Encyclopedia	Blautia wexlerae DSM 19850	DOE Joint Genome Institute (JGI)	2528768167	Firmicutes	Lachnospiraceae	1121115	DSM 19850
2857962555	Bacteria	Permanent Draft	Ruminococcus bromii G	Ruminococcus bromii ATCC 27255	University of Aberdeen	2857962555	Firmicutes	Oscillospiraceae	40518	ATCC 27255

Supplementary Table S3.2. Core species found in the literature

	Shetty et al. 2017	Faith et al. 2013	Qin et al. 2010	Falony et al. (2016)	Tap et al. 2009
	Top 50 taxa red = >90%	n=37 - LEAseq 16S		n= 1,106 >95%	n= 17 50% 10 most frequent OTUs
Akkermansia genus	x	x	x	x	
Alistipes genus	x	x	x	x	
Alistipes indictus	x	x			
Alistipes putredinis			x		x
Anaerococcus hydrogenalis		x			
Anaerostipes caccae	x				
Anaerostipes genus	x			x	
Anaerotruncus colihominis	x	x	x		
Anaerovorax odorimutans	x				
Bacteriodes xylanisolvens			x		
Bacteroides caccae		x	x		
Bacteroides capillosus			x		
Bacteroides cellulosilyicus			x		
Bacteroides coprocola			x		
Bacteroides coprophilus			x		
Bacteroides dorei		x	x		
Bacteroides eggerthii		x	x		
Bacteroides finegoldii			x		
Bacteroides fragilis	x		x		
Bacteroides genus	x	x	x	x	
Bacteroides intestinalis			x		
Bacteroides ovatus	x	x	x		
Bacteroides pectinophilus			x		
Bacteroides stercoris			x		x
Bacteroides thetaiotaomicron VPI-5482			x		
Bacteroides uniformis	x	x	x		
Bacteroides vulgatus	x	x	x		x
Barnesiella genus				x	
Bifidobacterium adolescentis			x		
Bifidobacterium genus	x	x	x	x	
Bifidobacterium longum			x		

Blautia genus	x	x	x	x	
Blautia hansenii			x		
Blautia obeum	x		x		x
Bryantella formatexigens	x				
Butyricicoccus genus				x	
Butyrivibrio crossotus	x		x		
Clostridium (sensu stricto)_ I	x				
Clostridium asparagiforme			x		
Clostridium bolteae			x		
Clostridium cellulosi	x				
Clostridium cluster XIVa	x			x	
Clostridium difficile	x				
Clostridium leptum	x	x	x		
Clostridium methylpentosum			x		
Clostridium nexile	x	x	x		
Clostridium scindens			x		
Clostridium sphenoides	x				
Clostridium stercorarium	x				
Clostridium symbiosum	x	x			
Clostridium_IV				x	
Clostridium_XI				x	
Collinsella aerofaciens			x		
Collinsella genus	x	x	x	x	
Coprococcus comes			x		
Coprococcus eutactus	x	x	x		
Coprococcus genus	x	x	x	x	
Dialister genus	x				
Dorea formicigenerans	x	x	x		
Dorea genus	x	x	x	x	
Dorea longicatena			x		x
Enterococcus faecalis			x		
Eubacterium hallii	x		x		x
Eubacterium rectale	x	x	x		x
Eubacterium siraeum			x		
Eubacterium ventriosum	x	x	x		
Faecalibacterium prausnitzii	x	x	x	x	x
Flavonifractor plautii	x				
Gemmiger				x	
Holdemania filiformis			x		
Lachnobacterium bovis	x				

Lachnospira pectinoschiza	x				
Lactobacillus genus		x			
Odoribacter genus	x			x	
Odoribacter splachnicus	x				
Oscillibacter genus	x			x	
Papillibacter cinnamivorans	x				
Parabacteroides distasonis	x		x		
Parabacteroides genus	x	x	x	x	
Parabacteroides johnsonii		x	x		
Parabacteroides merdae			x		
Prevotella genus	x		x	x	
Prevotella melaninogenica	x				
Prevotella oralis	x		x		
Roseburia genus	x	x	x	x	
Roseburia intestinalis	x	x	x		
Ruminococcus bromii	x		x		x
Ruminococcus callidus	x				
Ruminococcus genus	x	x	x	x	
Ruminococcus gnavus	x	x	x		
Ruminococcus lactaris	x	x	x		
Ruminococcus torques			x		
Sporobacter termitidis	x				
Streptococcus bovis	x				
Streptococcus genus	x		x	x	
Streptococcus mitis	x				
Streptococcus thermophilus			x		
Subdoligranulum variable	x	x	x		
Uncultured Clostridiales I	x				
Uncultured Clostridiales II	x				
Victivallis vadensis		x			

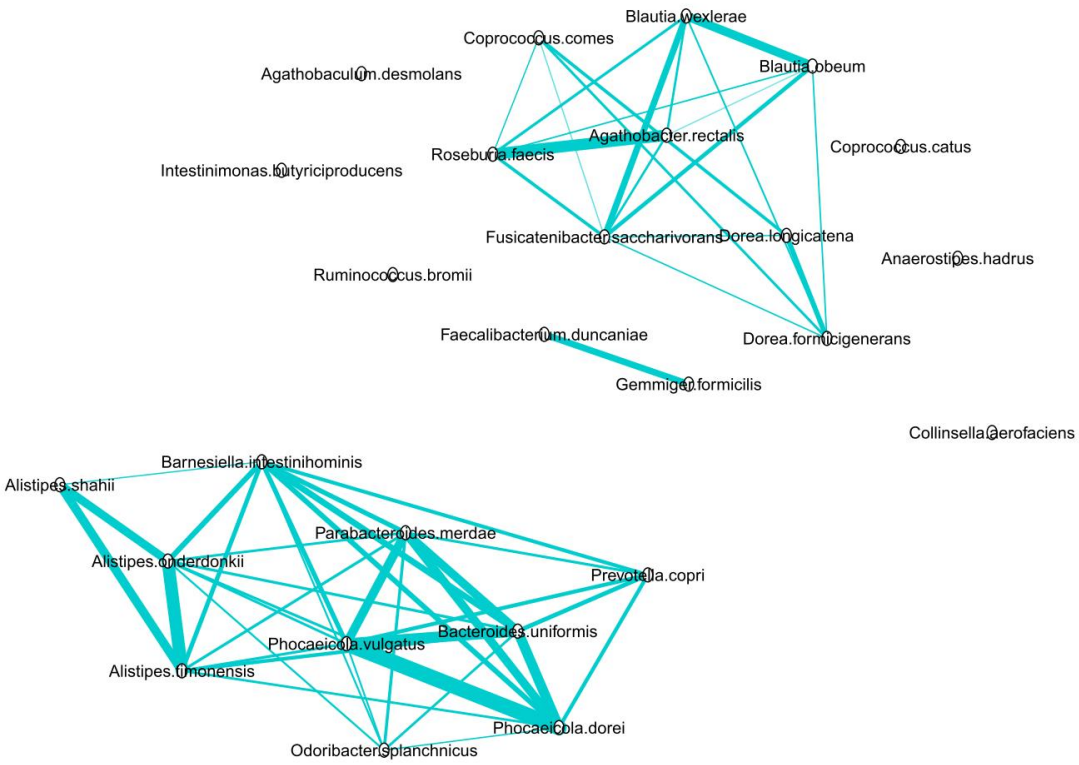
Supplementary Table S.3.3 A. Competition index matrix B. Cooperation index matrix

A.

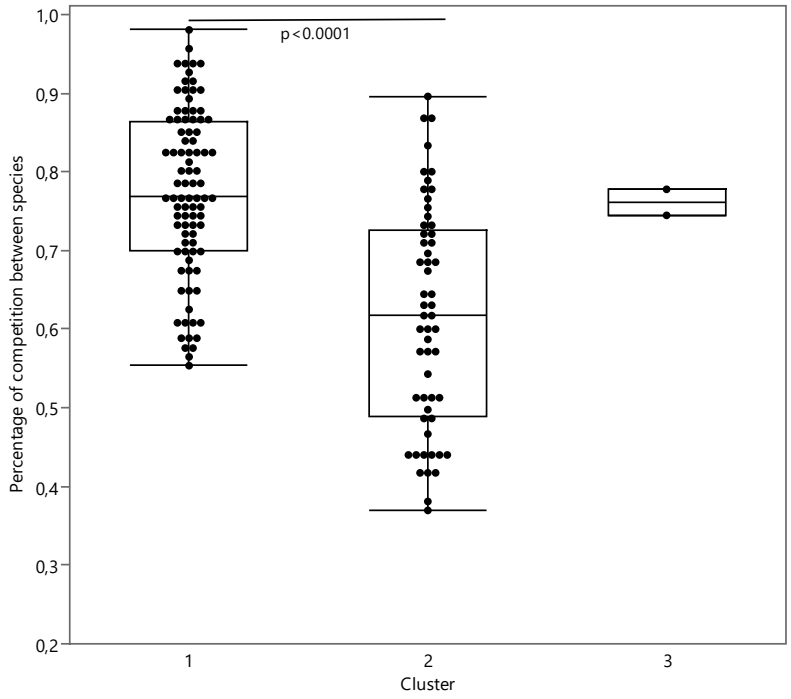
	A. hadrus	A. timoner	A. onderd	A. shahii	B. intestin	A. desmol	P. dorei	B. obeum	B. uniform	P. vulgatu	B. wexlera	C. catus	C. comes	C. aerofac	D. formici	D. longica	A. rectalis	F. duncani	F. sacchar	G. formici	I. butyripr	O. splanch	P. merdae	P. copri	R. bromii	R. faecis
A. hadrus	1	0.568627	0.431373	0.54902	0.607843	0.313725	0.411765	0.470588	0.470588	0.45098	0.666667	0.647059	0.411765	0.490196	0.54902	0.352941	0.54902	0.54902	0.45098	0.490196	0.54902	0.411765	0.431373	0.392157	0.333333	0.509804
A. timoner	0.517857	1	0.767857	0.767857	0.910714	0.285714	0.732143	0.517857	0.785714	0.75	0.660714	0.535714	0.446429	0.357143	0.428571	0.285714	0.535714	0.571429	0.517857	0.446429	0.482143	0.571429	0.767857	0.607143	0.232143	0.535714
A. onderd	0.478261	0.934783	1	0.76087	0.956522	0.347826	0.869565	0.630435	0.869565	0.630435	0.478261	0.543478	0.26087	0.347826	0.652174	0.630435	0.608696	0.543478	0.413043	0.695652	0.913043	0.76087	0.282609	0.673913		
A. shahii	0.608696	0.934783	0.76087	1	0.934783	0.26087	0.673913	0.543478	0.673913	0.695652	0.717391	0.565217	0.5	0.434783	0.478261	0.304348	0.565217	0.586957	0.543478	0.5	0.521739	0.586957	0.73913	0.652174	0.304348	0.565217
B. intestin	0.525424	0.864407	0.745763	0.728814	1	0.271186	0.745763	0.508475	0.728814	0.745763	0.644068	0.525424	0.440678	0.355932	0.423729	0.288136	0.525424	0.508475	0.508475	0.457627	0.474576	0.559322	0.728814	0.610169	0.220339	0.576271
A. desmol	0.64	0.64	0.64	0.48	0.64	1	0.52	0.8	0.64	0.6	0.84	0.72	0.6	0.36	0.56	0.36	0.8	0.72	0.6	0.68	0.6	0.6	0.64	0.48	0.56	0.76
P. dorei	0.411765	0.803922	0.784314	0.607843	0.862745	0.254902	1	0.568627	0.901961	0.980392	0.54902	0.411765	0.509804	0.215686	0.294118	0.333333	0.568627	0.529412	0.54902	0.470588	0.352941	0.647059	0.862745	0.647059	0.235294	0.588235
B. obeum	0.533333	0.644444	0.644444	0.555556	0.666667	0.444444	0.644444	1	0.666667	0.666667	0.866667	0.622222	0.6	0.311111	0.444444	0.444444	0.8	0.644444	0.644444	0.666667	0.422222	0.6	0.666667	0.6	0.377778	0.777778
B. uniform	0.428571	0.785714	0.714286	0.553571	0.767857	0.285714	0.821429	0.535714	1	0.839286	0.553571	0.410714	0.482143	0.232143	0.339286	0.321429	0.553571	0.571429	0.535714	0.464286	0.375	0.607143	0.75	0.625	0.285714	0.589286
P. vulgatu	0.410714	0.75	0.714286	0.571429	0.785714	0.267857	0.892857	0.535714	0.839286	1	0.517857	0.392857	0.482143	0.232143	0.303571	0.321429	0.535714	0.5	0.517857	0.446429	0.357143	0.589286	0.803571	0.589286	0.25	0.535714
B. wexlera	0.539683	0.587302	0.460317	0.52381	0.603175	0.333333	0.444444	0.619048	0.492063	0.460317	1	0.587302	0.492063	0.333333	0.444444	0.380952	0.68254	0.47619	0.571429	0.507937	0.412698	0.428571	0.460317	0.428571	0.285714	0.571429
C. catus	0.6875	0.625	0.458333	0.541667	0.645833	0.375	0.4375	0.583333	0.479167	0.458333	0.770833	1	0.416667	0.395833	0.604167	0.375	0.604167	0.479167	0.416667	0.4375	0.645833	0.479167	0.458333	0.375	0.354167	0.604167
C. comes	0.456522	0.543478	0.543478	0.5	0.565217	0.326087	0.565217	0.586957	0.586957	0.673913	0.434783	1	0.26087	0.434783	0.369565	0.73913	0.586957	0.695652	0.521739	0.391304	0.543478	0.586957	0.521739	0.26087	0.630435	
C. aerofac	0.641026	0.512821	0.307692	0.512821	0.538462	0.230769	0.282051	0.358974	0.333333	0.333333	0.538462	0.487179	0.307692	1	0.615385	0.333333	0.307692	0.410256	0.333333	0.410256	0.435897	0.282051	0.307692	0.307692	0.307692	0.333333
D. formici	0.692308	0.615385	0.410256	0.564103	0.641026	0.358974	0.384615	0.512821	0.487179	0.435897	0.717949	0.717949	0.512821	0.615385	1	0.615385	0.512821	0.512821	0.487179	0.410256	0.666667	0.487179	0.410256	0.461538	0.384615	0.512821
D. longica	0.514286	0.457143	0.457143	0.4	0.485714	0.257143	0.485714	0.571429	0.514286	0.514286	0.685714	0.514286	0.485714	0.371429	0.685714	1	0.6	0.542857	0.6	0.514286	0.428571	0.457143	0.457143	0.514286	0.257143	0.542857
A. rectalis	0.583333	0.625	0.625	0.541667	0.645833	0.416667	0.604167	0.75	0.645833	0.625	0.895833	0.604167	0.708333	0.25	0.416667	0.4375	1	0.666667	0.729167	0.625	0.4375	0.583333	0.625	0.583333	0.3125	0.833333
F. duncani	0.622222	0.711111	0.644444	0.6	0.666667	0.4	0.6	0.644444	0.711111	0.622222	0.666667	0.511111	0.6	0.355556	0.444444	0.422222	0.711111	1	0.622222	0.777778	0.444444	0.577778	0.644444	0.555556	0.266667	0.666667
F. sacchari	0.511111	0.644444	0.622222	0.555556	0.666667	0.333333	0.622222	0.644444	0.666667	0.644444	0.8	0.444444	0.711111	0.288889	0.422222	0.466667	0.777778	0.622222	1	0.555556	0.466667	0.555556	0.644444	0.6	0.288889	0.733333
G. formicil	0.531915	0.531915	0.489362	0.574468	0.361702	0.510638	0.638298	0.553191	0.531915	0.680851	0.446809	0.510638	0.446809	0.340426	0.340426	0.382979	0.638298	0.744681	0.531915	1	0.361702	0.468085	0.553191	0.489362	0.340426	0.659574
I. butyripr	0.622222	0.6	0.422222	0.533333	0.622222	0.333333	0.4	0.422222	0.466667	0.444444	0.577778	0.688889	0.4	0.377778	0.6	0.333333	0.466667	0.444444	0.466667	0.377778	1	0.4	0.444444	0.377778	0.355556	0.511111
O. splanch	0.525	0.8	0.8	0.675	0.825	0.375	0.825	0.675	0.85	0.825	0.675	0.575	0.625	0.275	0.475	0.4	0.7	0.65	0.625	0.55	0.45	1	0.825	0.7	0.35	0.725
P. merdae	0.458333	0.895833	0.875	0.708333	0.895833	0.333333	0.916667	0.625	0.875	0.9375	0.604167	0.458333	0.5625	0.25	0.333333	0.333333	0.625	0.604167	0.604167	0.541667	0.416667	0.6875	1	0.708333	0.270833	0.625
P. copri	0.5	0.85	0.875	0.75	0.9	0.3	0.825	0.675	0.875	0.825	0.675	0.45	0.6	0.3	0.425	0.425	0.7	0.625	0.675	0.575	0.425	0.7	0.85	1	0.325	0.75
R. bromii	0.586207	0.448276	0.448276	0.482759	0.448276	0.482759	0.413793	0.586207	0.551724	0.482759	0.62069	0.586207	0.413793	0.413793	0.517241	0.310345	0.517241	0.413793	0.448276	0.551724	0.551724	0.482759	0.448276	0.448276	1	0.551724
R. faecis	0.565217	0.652174	0.673913	0.565217	0.73913	0.413043	0.652174	0.76087	0.717391	0.652174	0.782609	0.630435	0.630435	0.282609	0.434783	0.413043	0.869565	0.652174	0.717391	0.673913	0.5	0.630435	0.652174	0.652174	0.347826	1

B.

	A. hadrus	A. timoner	A. onderd	A. shahii	B. intestin	A. desmol	P. dorei	B. obeum	B. uniform	P. vulgatu	B. wexlera	C. catus	C. comes	C. aerofac	D. formici	D. longica	A. rectalis	F. duncani	F. sacchar	G. formici	I. butyripr	O. splanch	P. merdae	P. copri	R. bromii	R. faecis
A. hadrus	0	0.176471	0.156863	0.137255	0.156863	0.058824	0.196078	0.098039	0.156863	0.176471	0.117647	0.117647	0.117647	0.078431	0.058824	0.078431	0.117647	0.078431	0.137255	0.117647	0.117647	0.137255	0.156863	0.137255	0.078431	0.098039
A. timoner	0.089286	0	0	0	0	0.017857	0.035714	0.071429	0.053571	0.035714	0.125	0.071429	0.089286	0.089286	0	0.053571	0.107143	0.035714	0.089286	0.071429	0.071429	0.017857	0	0.035714	0.035714	0.071429
A. onderd	0.108696	0.021739	0	0	0	0.021739	0.043478	0.086957	0.065217	0.043478	0.152174	0.086957	0.108696	0.108696	0	0.065217	0.130435	0.043478	0.108696	0.086957	0.065217	0.043478	0.021739	0	0.043478	0.086957
A. shahii	0.152174	0.065217	0	0	0.065217	0.086957	0.108696	0.130435	0.108696	0.195652	0.152174	0.173913	0.130435	0.108696	0.065217	0.130435	0.195652	0.108696	0.173913	0.130435	0.152174	0.086957	0.065217	0.065217	0.065217	0.152174
B. intestini	0.101695	0.033898	0.016949	0.016949	0	0.016949	0.033898	0.084746	0.050847	0.033898	0.135593	0.067797	0.084746	0.084746	0	0.050847	0.101695	0.033898	0.084746	0.067797	0.067797	0.033898	0.016949	0.016949	0.033898	0.067797
A. desmoli	0.12	0.08	0.08	0.08	0.12	0	0.24	0.08	0.16	0.2	0.08	0.2	0.16	0.16	0.08	0.12	0.12	0.12	0.12	0.08	0.12	0.16	0.12	0.16	0	0.12
P. dorei	0.078431	0.019608	0	0	0	0.019608	0	0.058824	0.058824	0	0.117647	0.078431	0.039216	0	0.039216	0.098039	0.019608	0.078431	0.058824	0.039216	0.039216	0.019608	0	0.019608	0	0.058824
B. uniform	0.111111	0.088889	0.088889	0.066667	0.066667	0.044444	0.155556	0	0.155556	0.155556	0.088889	0.111111	0.088889	0.044444	0.044444	0.044444	0.066667	0.044444	0.088889	0.066667	0.088889	0.088889	0.088889	0.044444	0.044444	0.066667
P. vulgatu	0.053571	0.035714	0.017857	0.017857	0.017857	0.035714	0.053571	0.053571	0	0.035714	0.089286	0.071429	0.053571	0	0.035714	0.089286	0.017857	0.071429	0.053571	0.035714	0.035714	0.035714	0.035714	0.035714	0.035714	0.053571
B. wexlerc	0.071429	0.017857	0.017857	0.017857	0.017857	0.035714	0.017857	0.071429	0.053571	0	0.125	0.089286	0.089286	0.035714	0	0.053571	0.107143	0.035714	0.089286	0.071429	0.035714	0.035714	0.017857	0.035714	0.035714	0.071429
C. catus	0.041667	0.083333	0.083333	0.083333	0.083333	0.041667	0.125	0.041667	0.104167	0.125	0.083333	0	0.083333	0.083333	0.041667	0	0.083333	0.041667	0.083333	0.083333	0.083333	0.104167	0.083333	0.041667	0.041667	0.041667
C. comes	0.173913	0.152174	0.152174	0.152174	0.23913	0.152174	0.195652	0.152174	0.173913	0.195652	0.152174	0.173913	0	0.195652	0.152174	0.173913	0.152174	0.152174	0.152174	0.130435	0.152174	0.26087	0.23913	0.152174	0.152174	0.152174
C. aerofac	0.230769	0.230769	0.230769	0.205128	0.230769	0.179487	0.25641	0.205128	0.230769	0.230769	0.230769	0.282051	0.230769	0	0.128205	0.179487	0.230769	0.205128	0.230769	0.179487	0.230769	0.205128	0.230769	0.230769	0.128205	0.230769
D. formici	0.25641	0.230769	0.230769	0.230769	0.230769	0.230769	0.307692	0.205128	0.230769	0.282051	0.205128	0.25641	0.205128	0.102564	0	0.076923	0.230769	0.205128	0.205128	0.230769	0.205128	0.230769	0.25641	0.179487	0.179487	0.230769
D. longica	0.285714	0.257143	0.257143	0.257143	0.257143	0.2	0.285714	0.2	0.285714	0.285714	0.228571	0.285714	0.171429	0.2	0.085714	0	0.2	0.257143	0.2	0.314286	0.228571	0.257143	0.285714	0.2	0.257143	0.228571
A. rectalis	0.0625	0.0625	0.041667	0.041667	0.041667	0.145833	0	0.104167	0.020833	0.083333	0.104167	0.0625	0	0.104167	0.020833	0.020833	0	0.0625	0.020833	0.083333	0.0625	0.145833	0.145833	0.020833	0.020833	0
F. duncani	0.066667	0.088889	0.066667	0.066667	0.066667	0.044444	0.133333	0.044444	0.111111	0.133333	0.088889	0.088889	0.111111	0.066667	0.022222	0.066667	0.088889	0	0.111111	0.022222	0.022222	0.066667	0.088889	0.088889	0.022222	0.044444
F. sacchari	0.044444	0.044444	0.044444	0.044444	0.133333	0.022222	0.111111	0.022222	0.088889	0.111111	0.022222	0.111111	0.022222	0.066667	0.044444	0.044444	0.022222	0.044444	0	0.044444	0.066667	0.155556	0.155556	0.066667	0.022222	0
G. formicil	0.148936	0.191489	0.170213	0.170213	0.170213	0.12766	0.234043	0.106383	0.191489	0.234043	0.12766	0.191489	0.148936	0.106383	0.12766	0.106383	0.148936	0.106383	0.170213	0	0.170213	0.148936	0.191489	0.170213	0.06383	0.12766
I. butyripr	0.044444	0.111111	0.111111	0.111111	0.111111	0.133333	0.066667	0.155556	0.044444	0.133333	0.155556	0.088889	0.111111	0.044444	0.066667	0.022222	0.111111	0.088889	0.044444	0.066667	0.066667	0	0.155556	0.111111	0.066667	0.044444
O. splanch	0.175	0.05	0.05	0.05	0.05	0.075	0.125	0.125	0.075	0.125	0.175	0.175	0.15	0.05	0.1	0.175	0.075	0.15	0.125	0.125	0	0.075	0.075	0.1	0.075	0.125
P. merdae	0.104167	0.020833	0.020833	0	0	0.020833	0.0625	0.0625	0.083333	0.0625	0.145833	0.083333	0.083333	0.083333	0	0.041667	0.104167	0.020833	0.104167	0.0625	0.041667	0.020833	0	0.020833	0.0625	
P. copri	0.175	0.075	0.05	0.05	0.05	0.05	0.125	0.15	0.125	0.125	0.1	0.125	0.1	0.15	0.025	0.1	0.125	0.075	0.1	0.15	0.125	0.1	0.1	0	0.075	0.125
R. bromii	0.206897	0.206897	0.206897	0.172414	0.241379	0.068966	0.344828	0.172414	0.241379	0.310345	0.172414	0.241379	0.206897	0.137931	0.103448	0.137931	0.206897	0.172414	0.172414	0.206897	0.241379	0.241379	0.206897	0	0.206897	
R. faecis	0.043478	0.108696	0.086957	0.086957	0.086957	0.043478	0.130435	0.043478	0.086957	0.130435	0.043478	0.086957	0.021739	0.065217	0.043478	0.043478	0.065217	0.043478	0.021739	0.043478	0.086957	0.108696	0.108696	0.065217	0.021739	0



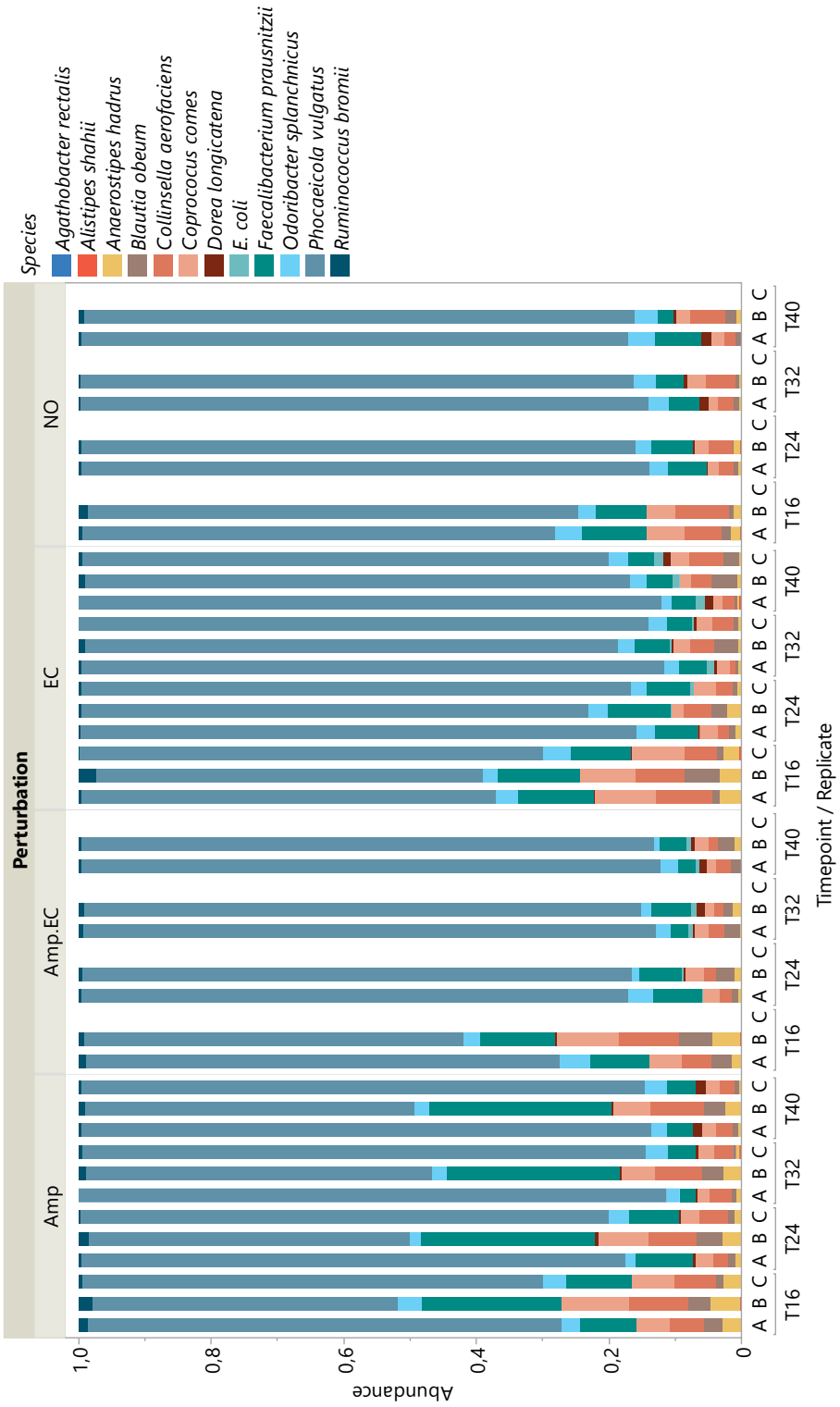
Supplementary Figure S3.1: Spearman correlation clusters from KO annotations from each genome ($\rho > 0.62$)



Supplementary Figure S3.2: Percentage of competition between each species per cluster (Kruskal-Wallis p <0.001, post hoc Dunn test $p < 0.0001$)

Supplementary material chapter 4

Supplementary Figure S4.1. Community composition at species level over time per perturbation with replicates shown.



Supplementary Table S4.1: Detailed selection of each HAEFC community member for the minimal community, taking into account health-associated studies, functional redundancy as well as competition, cooperation in silico analysis

Phylum	Species	Prevalence in B1 (genus level)	Prevalence in P (genus level)	Community Bact1/Prev	M-HAFC type	Species n°	Competition index	Cooperation index	Functional cluster	Key function (if relevant)	Health remarks
Actinobacteria	*Collinsella aerofaciens*	91%	95%	B1 / P	B1 / P	RCC1366	Lowest average index: 41%	21%	Not in cluster		
Bacteroidetes	*Alistipes onderdonkii*			B1/ P	-	-		4%			
Bacteroidetes	*Alistipes shahii*	100%	97%	B1/ P	B1	RCC662	High between the three species and with *P. copri* (>65%)	11%	All in cluster 1 *A. shahii* distinct profile from the other two species.		*A. shahii* has been observed to be decreased in liver cirrhosis patients and increased in positive responses of cancer therapy patients, and therefore seems to be positively associated with healthy conditions.
Bacteroidetes	*Alistipes timonensis*			B1/ P	-	-		5%			
Bacteroidetes	*Barnesiella intestinihominis*	90%	81%	B1	-	-	High competition index with *A. shahii* and *P. vulgatus* (72 and 74% respectively)	low complementary index on average (5%)	No extra functional potential (cluster 1)		
Bacteroidetes	*Odoribacter splanchnicus*	96%	94%	B1/ P	B1/P	RCC828	Low competition index for other species in the community (average of 62%)	11%	Cluster 1/ only species with succinate conversion potential		
Bacteroidetes	*Phocaeicola dorei*			B1	-	-	High between the three species (previously in same genus), within the community average competition *P. dorei* 57% while *P. vulgatus* 547%	4%	Cluster 1		
Bacteroidetes	*Bacteroides uniformis*	100%	100%	B1/ P	-	RCC1835		4%	Cluster 1		
Bacteroidetes	*Phocaeicola vulgatus*			B1/ P	B1	RCC1806		5%	Cluster 1		
Bacteroidetes	*Parabacteroides merdae*	98%	99%	B1/ P	-	-	High average competition (83%) with all cluster 1 members	5%	Cluster 1: No extra functional modules		Present in high abundance in hypertensive and polycystic ovary syndrome patients
Bacteroidetes	*Prevotella copri*	<60%	100%	P	P	DSM18205	65%	10%	Cluster 1		
Firmicutes	*Agathobacter rectalis*	100%	100%	B1/ P	B1/P	DSM17629	61%	6%	Cluster 2		
Firmicutes	*Roseburia faecis*			B1/ P	-	-	63%	7%	Cluster 2		
Firmicutes	*Anaerostipes hadrus*	100%	100%	B1/ P	B1/P	DSM3319	50%	12%	Not in cluster	Butyrate producer	
Firmicutes	*Blautia obeum*	100%	100%	B1/ P	B1/P	DSM25238	61%	*B. obeum* higher cooperation index (8% vs 6%)	Cluster 2		
Firmicutes	*Blautia wexlerae*			B1/ P	-	-	51%		Cluster 2		
Firmicutes	*Agathobaculum desmolans*	100%	100%	B1/ P	-	-	63%	12%	Not in cluster	Butyrate producer	
Firmicutes	*Coprococcus catus*	97%	100%	B1/ P	-	-	53%	6%	Not in cluster (less degradation of substrate compared to *C. comes*)		
Firmicutes	*Coprococcus comes*			B1/ P	B1/P	RCC3035	54%	16%	Cluster 2		
Firmicutes	*Intestinimonas butyriciproducens*	96%	95%	B1/ P	-	-	49%	8%	Not in cluster	Butyrate producer	
Firmicutes	*Dorea formicigenerans*	100%	100%	B1/ P	-	-	54%	*D. longicatena* = cooperation hub 23%, has 20% complementarity with 23 species (92% of species)	Cluster 2		
Firmicutes	*Dorea longicatena*			B1/ P	B1/P	DSM13814	51%		Cluster 2		
Firmicutes	*Fusicatenibacter saccharivorans*	100%	100%	B1/ P	-	-	59%	6%	Cluster 2	Formate conversion	
Firmicutes	*Faecalibacterium duncaniae*	100%	100%	B1/ P	B1/P	DSM17677T	60%	7%	Cluster 3	Butyrate producer, both dose functionally (only 2 in cluster 3)	*Faecalibacterium* = considered part of healthy microbiota and suggested as next gen probiotics, many research associated to probiotics
Firmicutes	*Gemmiger formicilis*	89%	99%	P	-	-	53%	15%	Cluster 3		
Firmicutes	*Ruminococcus bromii*	100%	100%	B1/ P	B1/P	RCC1377	50%	19%	Not in cluster	RS degrader, known to cross-feed with other bacteria (incl. *Agathobacter rectalis*)	

Supplementary Table S4.2: Media recipe for RCM, mGAM and the study medium MM++ including details on the reagents added into MM++.

Powder (g/L)	RCM	mGAM	MM++	Reagents for MM++
Agar	0.5			
D-Glucose	5	0.5	0.2	D-(+)-Glucose, Sigma-Aldrich
Digested serum		10		
Hemin		0.01	0.01	Hemin from bovine >=90%, Sigma-Aldrich
L-Arginine		1		
L-Cysteine HCl	0.5	0.3		
L-Tryptophan		0.2		
Liver extract		1.2		
Meat extract		2.2		
Mucin			2	Mucin from porcine stomach Type II, Sigma-Aldrich
Soya peptone		3		
Pectin			2	Citrus pectin 57% methyl esterification, LFT-SMART, KU Leuven, Belgium
Peptone	10	5		
Potassium dihydrogen phosphate (monobasic)		2.5		
Proteose peptone		5		
Sodium Acetate	3			
Sodium Bicarbonate			1.25	Sigma-Aldrich
Sodium Chloride	5	3	2.5	from LB (Luria Bertani Broth, Sigma-Aldrich)
Sodium thioglycollate		0.3		
Starch (soluble)	1	5	4	Starch from potato (soluble), Sigma-Aldrich
Tryptone			5	from LB (Luria Bertani Broth, Sigma-Aldrich)
Vitamin K (menadione)		0.005		
Xylan			1	≥95 % xylooligosaccharides, from corncob, Carl Roth
Yeast Extract	13	2.5	2.5	from LB (Luria Bertani Broth, Sigma-Aldrich)

Supplementary Table S4.3. Genomes accession number, taxonomy, completeness and contamination details of the custom database.

gtdb_accession	gtdb_taxonomy	checkm_completeness	checkm_contamination
GCF_000005845.2	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia;s__Escherichia coli	99.97	0.04
GCA_003463375.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	97.27	1.61
GCA_017986485.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	82.87	0.99
GCF_009019615.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.99
GCF_009019975.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.68
GCF_015557755.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_003464525.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	1.26
GCF_018289375.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_009019905.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_018060105.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	90.71	2.14
GCF_009020445.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_015553745.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_015679285.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_900217715.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_005845245.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_019131575.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.93
GCF_009020215.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_009020545.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_009020775.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_009020645.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_003472995.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_009019335.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_003436345.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	97.65	0.56
GCF_009020585.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_015552985.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_013361225.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.25
GCF_009019645.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_014871125.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	87.4	1.24
GCA_019012605.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	97.41	0.22
GCF_019130195.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_015552405.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.51	2.73
GCF_015558535.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5

GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
27915.1	Bacteroides;s__Bacteroides uniformis	98.88	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49845.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0179	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
86375.1	Bacteroides;s__Bacteroides uniformis	87.67	1.86
GCA_0148	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71175.1	Bacteroides;s__Bacteroides uniformis	96.77	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
50845.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20265.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60255.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68755.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19785.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30575.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19945.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20795.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0133	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
61195.1	Bacteroides;s__Bacteroides uniformis	99.24	0.37
GCA_0148	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71265.1	Bacteroides;s__Bacteroides uniformis	97.49	0.74
GCF_0002	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
73275.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCA_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02845.1	Bacteroides;s__Bacteroides uniformis	98.61	0.12
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
82075.1	Bacteroides;s__Bacteroides uniformis	99.24	0.02
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29075.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69475.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29685.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
54205.1	Bacteroides;s__Bacteroides uniformis	98.88	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39225.1	Bacteroides;s__Bacteroides uniformis	99.26	0.81
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59625.1	Bacteroides;s__Bacteroides uniformis	99.26	0.87
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28965.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29135.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03995.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
54825.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60385.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30605.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
92165.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
37605.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19055.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20275.1	Bacteroides;s__Bacteroides uniformis	99.26	1.63
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20805.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19585.1	Bacteroides;s__Bacteroides uniformis	99.26	0

GCF_0036 03065.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0036 02965.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.62
GCA_0035 34475.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	85.28	0.12
GCF_0034 38145.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034 69415.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0090 19085.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0151 24985.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCA_0035 31795.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	73.45	0
GCF_0090 19735.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191 28055.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0155 57045.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191 28275.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.88	0
GCF_0034 57925.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0090 19245.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0001 62215.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090 20465.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0090 20635.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0034 38985.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.07	0.31
GCF_0034 60085.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034 70495.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.68
GCF_0090 19135.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090 19075.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0035 39575.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	85.13	0.74
GCF_0090 19105.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090 20375.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_0090 20165.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	9.08
GCF_0090 20115.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.58
GCF_0138 74375.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	2.62
GCF_0155 48005.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_0036 02755.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.87
GCA_0148 71105.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.15	2.38
GCF_0090 19535.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0155 55535.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0002 73785.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0156 71055.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.76	1.63

GCA_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
65735.1	Bacteroides;s__Bacteroides uniformis	94.6	0
GCF_0142	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
87575.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28995.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29055.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39715.1	Bacteroides;s__Bacteroides uniformis	98.22	1.3
GCA_0148	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71205.1	Bacteroides;s__Bacteroides uniformis	94.38	0.12
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02735.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19145.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19695.1	Bacteroides;s__Bacteroides uniformis	99.26	0.72
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19935.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03955.1	Bacteroides;s__Bacteroides uniformis	99.19	9.21
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20535.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
70725.1	Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28475.1	Bacteroides;s__Bacteroides uniformis	98.88	0
GCA_0178	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51235.1	Bacteroides;s__Bacteroides uniformis	97.4	0.95
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20735.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
06135.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19445.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
27755.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20255.1	Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32675.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03545.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03015.1	Bacteroides;s__Bacteroides uniformis	99.26	1.64
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
70605.1	Bacteroides;s__Bacteroides uniformis	99.04	1.18
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69845.1	Bacteroides;s__Bacteroides uniformis	98.01	2.21
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
36255.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20005.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03375.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19775.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59965.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20575.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0009	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39155.1	Bacteroides;s__Bacteroides uniformis	99.19	9.21
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02895.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20315.1	Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20175.1	Bacteroides;s__Bacteroides uniformis	99.26	0.19

GCF_009019355.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_000185585.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.39	0.37
GCF_009019655.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.35	0.74
GCF_015559225.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_005844805.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_015551105.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_015668775.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCA_014871475.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	97.67	1.56
GCF_019127825.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.88	0
GCA_003474125.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	97.73	0.19
GCF_003603175.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_009020475.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_009019825.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_018704075.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	86.25	5.33
GCF_003468325.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_009019385.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_009019345.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_003469025.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.99
GCF_009020405.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_900896415.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	1.9
GCF_003471545.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCA_008679735.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.88	3.53
GCA_014871165.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	87.71	4.15
GCF_000699885.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	12.7
GCF_009020055.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.68
GCF_009019275.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.55
GCF_019130175.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_015547185.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_003459325.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_009019635.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.13	0.37
GCF_019128355.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.88	0
GCA_008680795.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	96.45	2.23
GCF_003475375.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_009019455.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_014871195.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	93.35	0

GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19545.1	Bacteroides;s__Bacteroides uniformis	99.07	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
94615.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20435.1	Bacteroides;s__Bacteroides uniformis	91.85	1.22
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
50455.1	Bacteroides;s__Bacteroides uniformis	99.26	1.3
GCF_0067	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
42345.1	Bacteroides;s__Bacteroides uniformis	99.26	0.87
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
61145.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_9028	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25465.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
52455.1	Bacteroides;s__Bacteroides uniformis	98.88	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19805.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03955.1	Bacteroides;s__Bacteroides uniformis	99.26	0.31
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20815.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
48415.1	Bacteroides;s__Bacteroides uniformis	62.64	0.37
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20685.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCA_0023	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20565.1	Bacteroides;s__Bacteroides uniformis	97.24	0.12
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
06095.1	Bacteroides;s__Bacteroides uniformis	97.77	0.09
GCA_0180	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
54275.1	Bacteroides;s__Bacteroides uniformis	77.93	1.72
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69465.1	Bacteroides;s__Bacteroides uniformis	99.26	0.99
GCF_9001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
07315.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
87475.1	Bacteroides;s__Bacteroides uniformis	94.54	0.37
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03935.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19885.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
52515.1	Bacteroides;s__Bacteroides uniformis	92.19	0.56
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19185.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19435.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32285.1	Bacteroides;s__Bacteroides uniformis	98.88	0.12
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02695.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0006	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
99825.1	Bacteroides;s__Bacteroides uniformis	97.73	6.91
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
66325.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03175.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20365.1	Bacteroides;s__Bacteroides uniformis	97.4	0.95
GCA_9105	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
83705.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28015.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67505.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
55865.1	Bacteroides;s__Bacteroides uniformis	99.26	1.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20035.1	Bacteroides;s__Bacteroides uniformis	99.26	0.68

GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49605.1	Bacteroides;s__Bacteroides uniformis	99.26	0.87
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68465.1	Bacteroides;s__Bacteroides uniformis	97.65	0.12
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
50345.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0187	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
85825.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20455.1	Bacteroides;s__Bacteroides uniformis	99.26	3.29
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28575.1	Bacteroides;s__Bacteroides uniformis	98.88	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19475.1	Bacteroides;s__Bacteroides uniformis	98.7	0.74
GCF_9006	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
24705.1	Bacteroides;s__Bacteroides uniformis	99.26	0.43
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39055.1	Bacteroides;s__Bacteroides uniformis	90.52	3.35
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
64785.1	Bacteroides;s__Bacteroides uniformis	99.26	1.61
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
37005.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19195.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19725.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0180	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
58485.1	Bacteroides;s__Bacteroides uniformis	88.15	1.49
GCF_0058	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
44235.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20755.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
38465.1	Bacteroides;s__Bacteroides uniformis	99.07	2.91
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69435.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29315.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0148	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71095.1	Bacteroides;s__Bacteroides uniformis	97.82	0.37
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68465.1	Bacteroides;s__Bacteroides uniformis	98.39	2.71
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02945.1	Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20675.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05475.1	Bacteroides;s__Bacteroides uniformis	98.51	0.37
GCF_0015	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
78555.1	Bacteroides;s__Bacteroides uniformis	98.88	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30215.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03315.1	Bacteroides;s__Bacteroides uniformis	99.26	1.3
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19835.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68865.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0161	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
17815.1	Bacteroides;s__Bacteroides uniformis	99.26	1.12
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
50905.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_9023	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74035.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20155.1	Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20705.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03055.1	Bacteroides;s__Bacteroides uniformis	99.26	1.12

GCF_009019315.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_905197615.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_009020605.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_015124975.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_001406715.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	98.51	0.68
GCF_009020355.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_018785865.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_019130685.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCA_017996295.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	77.43	2.84
GCF_003463315.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_018291785.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_004167505.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_009020225.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.19
GCA_900185075.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	96.09	0.25
GCF_019129195.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_009020135.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_003603515.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCA_018181015.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_003466885.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.87
GCF_003602795.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_009019875.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_009739405.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	96	0.74
GCF_009019985.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_015560645.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_003603275.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.93
GCF_009019555.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_003439685.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.81
GCF_009020325.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	5.24
GCF_003603495.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_003466245.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_019132605.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_003603665.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.12
GCF_003438205.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_017620935.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	87.49	2.63
GCF_009020045.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides uniformis	99.26	0.5

GCF_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
89175.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59585.1	Bacteroides;s__Bacteroides uniformis	99.26	0.37
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19235.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0021	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
61435.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
37295.1	Bacteroides;s__Bacteroides uniformis	97.53	0.12
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59845.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
75315.1	Bacteroides;s__Bacteroides uniformis	99.06	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49665.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03395.1	Bacteroides;s__Bacteroides uniformis	99.26	1.12
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19395.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
73285.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02995.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05595.1	Bacteroides;s__Bacteroides uniformis	98.88	1.24
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51575.1	Bacteroides;s__Bacteroides uniformis	99.26	0.74
GCF_0043	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28515.1	Bacteroides;s__Bacteroides uniformis	99.26	0.56
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
36475.1	Bacteroides;s__Bacteroides uniformis	99.26	1.05
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32075.1	Bacteroides;s__Bacteroides uniformis	99.26	0.93
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
20075.1	Bacteroides;s__Bacteroides uniformis	99.26	0.5
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29335.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
19285.1	Bacteroides;s__Bacteroides uniformis	99.26	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69535.1	Bacteroides;s__Bacteroides uniformis	98.05	0.31
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
06635.1	Bacteroides;s__Bacteroides uniformis	98.88	0.12
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
37205.1	Bacteroides;s__Bacteroides uniformis	99.26	0.68
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
63925.1	Bacteroides;s__Bacteroides uniformis	98.33	0.12
GCF_0004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03155.2	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
70025.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.18
GCA_9051	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
93355.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0083	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29425.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.86
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31255.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68615.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.4	0.57
GCF_0043	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49635.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.67
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
46775.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31955.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.08
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
06075.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.03	0.24
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59985.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4

GCF_9001 09965.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.08
GCA_0010 49555.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	98.93	3.52
GCF_0191 31685.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.08
GCF_0155 56465.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.13
GCF_0155 47465.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0167 60085.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.19
GCF_0156 70015.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	97.47	0.67
GCF_0155 54035.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	1.11
GCF_0191 32235.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.32
GCA_0004 37995.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	78.45	0
GCF_0155 51275.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCF_0191 26395.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.67
GCF_0090 25475.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0034 34395.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.18
GCA_0034 74005.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	97.99	0
GCF_9004 45585.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.3	1.81
GCA_0148 71295.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.73
GCF_0034 58585.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.25	0.32
GCF_0167 60065.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.99
GCF_0191 32245.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	98.81	0.08
GCF_0010 49535.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.37	0.81
GCF_0155 54205.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.14	0.24
GCF_0156 70455.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	98.81	2.37
GCF_0155 48485.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.3	0.4
GCF_9004 45595.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	98.79	1.08
GCA_0034 63855.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	96.79	0.24
GCF_0155 48265.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.44
GCF_0191 32635.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCF_0161 03195.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	2.02
GCF_0167 60165.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.78
GCF_0155 56535.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	98.98	0.22
GCF_0155 55485.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.88
GCF_0034 69365.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0191 31205.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0034 57815.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0

GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30675.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.43
GCF_9001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
09385.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
72675.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68465.1	Bacteroides;s__Bacteroides thetaiotaomicron	98.79	0.9
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29275.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.32
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
52485.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.14	0.2
GCF_0000	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
11065.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59925.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	1.09
GCF_0187	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
85885.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.4
GCF_0168	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
88545.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59875.2	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
56665.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.13
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60095.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	2.37
GCF_9001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
08155.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0187	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
85895.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30475.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.43
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31235.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0161	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
17715.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.3	0.08
GCF_0083	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29455.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	1.16
GCA_0001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
62515.1	Bacteroides;s__Bacteroides thetaiotaomicron	98.45	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
63095.1	Bacteroides;s__Bacteroides thetaiotaomicron	97.51	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25525.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.67
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51985.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.39
GCA_0062	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
18125.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	4.91
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30355.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
64695.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.16
GCF_0015	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
78565.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0158	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31785.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60015.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	3.31
GCF_0043	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49615.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.3	1.05
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68095.1	Bacteroides;s__Bacteroides thetaiotaomicron	98.69	0.65
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29915.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.43
GCF_0121	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51035.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39845.1	Bacteroides;s__Bacteroides thetaiotaomicron	96.91	0.59
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74715.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.31	0.73

GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41315.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.32
GCF_9023	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
64285.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69685.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32575.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32455.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCF_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
91825.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.35	0.08
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
47765.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.16
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25795.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.67
GCF_0158	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31845.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25275.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39015.1	Bacteroides;s__Bacteroides thetaiotaomicron	57.76	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
72055.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.16
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25395.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30435.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.57
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60105.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	1.03
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
53025.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.19
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41325.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.32
GCF_0029	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59695.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.25	0.73
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30375.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30755.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32815.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
66025.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.46	0.49
GCF_0018	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
16245.2	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.19
GCF_0010	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
55755.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.59
GCF_0013	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
14975.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60505.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.08
GCF_9006	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
24795.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0075	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
56425.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.83
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05255.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25625.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.67
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25335.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.24
GCF_0141	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31755.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05895.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.09	0.3
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30495.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.43
GCF_0041	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67525.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.57

GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32695.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60005.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	1.63
GCF_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
89275.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.4
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
48225.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.08
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25725.1	Bacteroides;s__Bacteroides thetaiotaomicron	99.41	0.67
GCF_0001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
57935.1	Prevotella;s__Prevotella copri	98.31	1.35
GCF_0007	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
01705.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	100	0
GCA_0180	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
56005.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	77.51	2.47
GCF_0001	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
85705.2	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	100	0
GCA_0183	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
67825.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	100	0
GCA_9052	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
11375.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	100	0
GCF_9023	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
74275.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	100	0
GCA_0183	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
68125.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	97.63	0
GCF_0002	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
24655.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	100	0
GCA_0182	d__Bacteria;p__Desulfobacterota_I;c__Desulfovibrionia;o__Desulfovibrionales;f__Des		
65765.1	ulfovibrionaceae;g__Bilophila;s__Bilophila wadsworthia	95.13	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
36855.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28155.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29555.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0006	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
99705.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0000	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
12825.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.94
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
38645.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
82925.1	Phocaeicola;s__Phocaeicola vulgatus	79.32	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41435.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28415.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74145.1	Phocaeicola;s__Phocaeicola vulgatus	97.22	0.19
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41535.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
73085.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0075	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
56395.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.39
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67095.1	Phocaeicola;s__Phocaeicola vulgatus	97.7	0
GCA_0148	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49105.1	Phocaeicola;s__Phocaeicola vulgatus	71.36	3.38
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
62865.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
64405.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.31
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74985.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
55055.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
02805.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0

GCF_0004 03235.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129 76275.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191 26015.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155 53265.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191 29605.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0155 47385.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155 56625.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0182 65455.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	92.72	0.5
GCF_0155 55885.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 26025.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0006 99845.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0155 57505.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.15	1.92
GCF_0191 29895.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 26755.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190 61335.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0058 44385.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 30535.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 72125.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 57755.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.31
GCA_0045 58735.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	86.72	4.48
GCF_0191 30115.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 71775.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155 61305.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 65015.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129 76375.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155 52815.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 75435.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.66
GCF_0191 28715.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 32215.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCA_0034 63605.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	97.99	0.19
GCF_0155 59705.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0001 58515.2	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0156 67615.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0183 65615.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	93.79	1.09
GCA_0004 38035.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	96.91	0

GCA_0034 66665.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	97.23	0.19
GCF_0191 28325.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0034 38705.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0184 92915.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129 76285.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0001 86105.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.82
GCA_0097 75675.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155 47315.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 30515.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 31275.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 59715.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191 25895.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 28815.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_9004 45545.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0034 63745.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	98.54	0.5
GCF_0034 65765.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0034 65305.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0035 68805.2	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.2
GCF_0029 59625.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_0034 75135.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 74595.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034 73265.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.56
GCF_0034 66785.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0156 67825.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.15	1.54
GCF_0155 50745.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0156 68105.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0191 28615.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0155 57255.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0187 85805.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191 31875.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.25
GCF_0078 96815.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 26365.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191 29475.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCA_0024 93975.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	72.76	0
GCF_0058 44725.1	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Phocaeicola;s__Phocaeicola vulgatus	99.25	0

GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29995.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32435.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
64205.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0187	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
85835.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.56
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59475.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03775.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0019	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
15605.1	Phocaeicola;s__Phocaeicola vulgatus	96.6	0.75
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41515.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCA_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
13185.1	Phocaeicola;s__Phocaeicola vulgatus	94.74	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
27895.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39605.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
70745.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0027	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
96815.1	Phocaeicola;s__Phocaeicola vulgatus	99.15	0
GCF_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
89355.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51975.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28545.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0006	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
99865.1	Phocaeicola;s__Phocaeicola vulgatus	98.49	1.32
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41415.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25635.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41855.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71705.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76765.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29485.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCA_0086	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
80875.1	Phocaeicola;s__Phocaeicola vulgatus	96.6	1.04
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60125.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25385.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0086	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
79395.1	Phocaeicola;s__Phocaeicola vulgatus	92.46	3.82
GCF_0078	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
96765.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28755.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29795.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26475.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
52755.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCA_0086	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
80195.1	Phocaeicola;s__Phocaeicola vulgatus	97.37	0.56
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
56815.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_9001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
09635.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0

GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
63885.1	Phocaeicola;s__Phocaeicola vulgatus	98.12	0.38
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25375.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59725.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76775.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41815.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_9105	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76625.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
65695.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
38765.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
87465.1	Phocaeicola;s__Phocaeicola vulgatus	65.05	0.02
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
75695.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
13025.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.69
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29575.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74845.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
42075.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
42015.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.5
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26455.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28135.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0148	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71015.1	Phocaeicola;s__Phocaeicola vulgatus	99.22	0.45
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
72225.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30915.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
12315.1	Phocaeicola;s__Phocaeicola vulgatus	97.59	0.13
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29925.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0041	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67625.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.31
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68905.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
37415.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25805.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41715.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68525.1	Phocaeicola;s__Phocaeicola vulgatus	98.87	1.74
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41475.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41655.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.94
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30925.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76295.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.56
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30095.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0133	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68705.1	Phocaeicola;s__Phocaeicola vulgatus	99.22	4.13
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
58385.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38

GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
42195.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.01
GCF_0133	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
61175.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0036	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
03635.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0002	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
73295.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
38235.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25675.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
06315.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68485.1	Phocaeicola;s__Phocaeicola vulgatus	98.36	0.57
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25995.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29595.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29675.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68545.1	Phocaeicola;s__Phocaeicola vulgatus	98.58	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41605.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_9105	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
73775.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28315.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28655.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
58265.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
04375.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
57705.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68345.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28745.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
57525.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31715.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
55305.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
07255.1	Phocaeicola;s__Phocaeicola vulgatus	69.66	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41755.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.69
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67565.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0156	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67255.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
61035.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCA_9007	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51965.1	Phocaeicola;s__Phocaeicola vulgatus	74.51	2.62
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28075.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29355.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
66935.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
68295.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0087	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28395.1	Phocaeicola;s__Phocaeicola vulgatus	98.87	0.5

GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76785.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29515.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
36605.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26435.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0090	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25535.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.25
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59535.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.25
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69525.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76385.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
75835.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74155.1	Phocaeicola;s__Phocaeicola vulgatus	98.5	0.38
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
55435.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26045.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0086	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
72705.1	Phocaeicola;s__Phocaeicola vulgatus	91.71	5.28
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
67465.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCA_0178	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
51375.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	1.13
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25945.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26095.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
72865.1	Phocaeicola;s__Phocaeicola vulgatus	98.3	0.19
GCF_0001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
78195.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28085.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
36175.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0190	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
41765.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.57
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
55645.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCA_0179	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
88495.1	Phocaeicola;s__Phocaeicola vulgatus	70.69	1.72
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
27815.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29635.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29775.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31105.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76745.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
48125.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_9007	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
61055.1	Phocaeicola;s__Phocaeicola vulgatus	62.89	0.86
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
96545.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.5
GCF_0032	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
07995.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39595.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69035.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0

GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71285.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0129	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
76365.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
69225.1	Phocaeicola;s__Phocaeicola vulgatus	98.87	0
GCA_0019	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
13895.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
49725.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28215.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
37875.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0167	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
66915.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.94
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
32155.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26135.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
75045.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
30815.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
71225.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_9052	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05605.1	Phocaeicola;s__Phocaeicola vulgatus	99.62	0.01
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
66945.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
74225.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
86545.1	Phocaeicola;s__Phocaeicola vulgatus	94.23	1.13
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59115.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
29535.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.38
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
28665.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.75
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
38835.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.13
GCF_0075	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
56405.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.39
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
52105.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
39275.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
26235.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
58775.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
25935.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
60305.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0078	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
96665.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
59365.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	1.51
GCF_0014	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05155.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.19
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
54325.1	Phocaeicola;s__Phocaeicola vulgatus	99.25	0.56
GCA_0002	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
09935.1	_Agathobacter;s__Agathobacter rectalis	99.28	1.21
GCF_0002	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
10695.1	_Anaerostipes;s__Anaerostipes hadrus	98.66	1.34
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
74055.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0

GCF_0028	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
34235.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
67645.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
63925.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_9002	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
91485.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.68
GCA_0035	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
35095.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
61945.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0182	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
65955.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCA_0183	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
79655.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
69265.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
72955.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCA_0024	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
93255.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	92.79	2.18
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
74265.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	92.62	0.67
GCA_9023	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
64845.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68785.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
67885.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	1.01
GCA_9052	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
04595.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
62345.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
70075.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	95.08	1.81
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
63975.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	86.17	1.01
GCA_0019	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
17065.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	76.58	0.67
GCA_0035	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
34835.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68245.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
79975.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	90.49	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
61485.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
86865.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	96.64	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
73275.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.99	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
66225.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68015.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0023	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
63275.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	77.4	0
GCF_0097	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
18335.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCF_0097	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
18325.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
35955.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.99	0.67
GCF_0028	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
34165.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.68
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
61985.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.81
GCA_0183	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68635.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67

GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
61435.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
80445.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCA_0179	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
86845.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	80.03	1.01
GCA_0023	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
61965.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCA_9000	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
67015.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
63985.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.99	1.34
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
62015.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0004	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
33495.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
66205.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
74425.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
64295.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
62185.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.8	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
70125.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
59405.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	96.74	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
73075.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
66725.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.32	1.01
GCF_0187	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
83565.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
62025.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
63575.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.99	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
70105.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68955.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
60445.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
70065.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	2.8
GCF_0097	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
18385.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
70165.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.66	0.67
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
66165.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
67435.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0180	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
53305.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	65.52	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
58325.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.26	0.67
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
70135.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	93.96	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
35865.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	98.3	1.01
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68645.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
62205.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
62335.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	0.67
GCA_0009	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
80315.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	97.32	3.02

GCA_0086	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
68465.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_0179	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
95715.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	74.14	3.45
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
50625.1	__Ruminococcus_E;s__Ruminococcus_E bromii_B	99.33	1.01
GCA_9000	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
66425.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
62725.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
56105.1	_Bariatricus;s__Bariatricus comes	99.42	1
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01525.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0132	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
99745.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
50485.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCA_0189	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
16865.1	_Bariatricus;s__Bariatricus comes	78.95	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01515.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0187	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
85205.1	_Bariatricus;s__Bariatricus comes	99.42	0.39
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
52615.1	_Bariatricus;s__Bariatricus comes	99.42	0.39
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
60155.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0132	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
99545.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00805.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
52445.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0014	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
06395.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
53595.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
48735.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0132	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
99665.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00635.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
48425.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
56185.1	_Bariatricus;s__Bariatricus comes	99.42	0.29
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
54465.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
65255.1	_Bariatricus;s__Bariatricus comes	94.93	4.43
GCF_0187	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
85285.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01475.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0156	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
71025.1	_Bariatricus;s__Bariatricus comes	99.42	1.17
GCF_0132	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
99925.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCA_0004	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
36715.1	_Bariatricus;s__Bariatricus comes	98.54	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
36145.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0014	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
04595.1	_Bariatricus;s__Bariatricus comes	99.22	0.12
GCA_0183	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
64175.1	_Bariatricus;s__Bariatricus comes	99.22	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01605.1	_Bariatricus;s__Bariatricus comes	99.42	0

GCF_0132	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
99755.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00605.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
47305.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
58505.1	_Bariatricus;s__Bariatricus comes	99.42	0.39
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
54215.1	_Bariatricus;s__Bariatricus comes	99.42	0.88
GCA_0001	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
55875.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0132	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
99685.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0014	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
05795.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00315.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00745.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0014	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
04815.1	_Bariatricus;s__Bariatricus comes	99.42	0.39
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00465.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
71185.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01545.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
61125.1	_Bariatricus;s__Bariatricus comes	99.42	0.1
GCF_0187	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
85165.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCA_9023	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
73925.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
60315.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00095.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCA_9051	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
92695.1	_Bariatricus;s__Bariatricus comes	99.42	0.64
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01505.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01565.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
01465.1	_Bariatricus;s__Bariatricus comes	99.42	0.19
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
54275.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0169	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
04155.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0034	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
70335.1	_Bariatricus;s__Bariatricus comes	99.42	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
32345.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
65155.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_9023	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
64825.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCA_0179	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
97005.1	Odoribacter;s__Odoribacter splanchnicus	83.87	1.08
GCA_0009	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
80245.1	Odoribacter;s__Odoribacter splanchnicus	98.83	0
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77565.1	Odoribacter;s__Odoribacter splanchnicus	97.85	0.54
GCA_0019	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
15655.1	Odoribacter;s__Odoribacter splanchnicus	90.86	0.54
GCF_0001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
90535.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
66925.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0.54

GCF_0021	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
61635.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0.54
GCA_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77945.1	Odoribacter;s__Odoribacter splanchnicus	99.46	1.88
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
42695.1	Odoribacter;s__Odoribacter splanchnicus	94.62	0
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
16405.1	Odoribacter;s__Odoribacter splanchnicus	87.06	0.27
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77915.1	Odoribacter;s__Odoribacter splanchnicus	90.59	3.24
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
32715.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
34845.1	Odoribacter;s__Odoribacter splanchnicus	90.86	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
63405.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
69405.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
53175.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0.54
GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
48135.1	Odoribacter;s__Odoribacter splanchnicus	81.63	0
GCF_0133	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
61165.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
32795.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
54525.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
62805.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
67205.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
53905.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
78805.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
49625.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
51685.1	Odoribacter;s__Odoribacter splanchnicus	98.92	0.91
GCA_0004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
35735.1	Odoribacter;s__Odoribacter splanchnicus	96.51	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
49315.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77575.1	Odoribacter;s__Odoribacter splanchnicus	99.46	1.23
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77835.1	Odoribacter;s__Odoribacter splanchnicus	98.92	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
64465.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0.54
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77975.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
78795.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_9004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
54195.1	Odoribacter;s__Odoribacter splanchnicus	99.11	0.18
GCF_0021	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
59415.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCA_0180	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
55845.1	Odoribacter;s__Odoribacter splanchnicus	79.31	1.72
GCF_0187	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
84285.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
32315.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
74785.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0096	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
77935.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_9001	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
87175.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0

GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
60605.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
54715.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCA_9052	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
04195.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
46895.1	Odoribacter;s__Odoribacter splanchnicus	99.46	0
GCA_0035	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
29505.1	Odoribacter;s__Odoribacter splanchnicus	90.86	0
GCA_0182	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
65795.1	Odoribacter;s__Odoribacter splanchnicus	95.16	0.54
GCA_0009	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae;g__		
80295.1	Odoribacter;s__Odoribacter splanchnicus	98.56	0
GCF_0001	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
54065.1	_Dorea_A;s__Dorea_A longicatena	98.83	0
GCF_0027	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g_		
34145.1	_Faecalibacterium;s__Faecalibacterium prausnitzii_C	100	0
GCF_0001	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
53905.1	_Blautia_A;s__Blautia_A obeum	99.01	0
GCA_9007	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
59165.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	70.93	1.01
GCF_0156	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
70765.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	99.19	0.81
GCF_0058	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
44325.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75975.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
03675.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76015.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76125.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76075.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
47365.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
15875.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	99.19	0.92
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
13445.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
42955.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	97.58	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76065.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCA_0148	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
71675.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	96.77	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
74215.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76155.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	97.98	2.69
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
78115.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	92.2	3.63
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76045.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCA_9007	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
53075.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	96.29	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76025.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_G	100	0.81
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
56995.1	istipes;s__Alistipes shahii	100	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
47645.1	istipes;s__Alistipes shahii	100	0.48
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
59775.1	istipes;s__Alistipes shahii	100	0
GCF_0191	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
32105.1	istipes;s__Alistipes shahii	100	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
56735.1	istipes;s__Alistipes shahii	100	0.48

GCA_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
89785.1	istipes;s__Alistipes shahii	56.03	0
GCF_0155	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
58405.1	istipes;s__Alistipes shahii	100	0
GCA_0183	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
68275.1	istipes;s__Alistipes shahii	98.99	0
GCF_0034	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
61645.1	istipes;s__Alistipes shahii	100	0
GCF_0084	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
16365.1	istipes;s__Alistipes shahii	100	0.51
GCF_0084	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
16385.1	istipes;s__Alistipes shahii	100	0
GCF_0002	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
10575.1	istipes;s__Alistipes shahii	97.12	0
GCA_0179	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
37585.1	istipes;s__Alistipes shahii	98.32	0.72
GCA_9052	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
00485.1	istipes;s__Alistipes shahii	100	0
GCA_0019	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
15285.1	istipes;s__Alistipes shahii	95.9	1.22
GCF_0128	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
44205.1	istipes;s__Alistipes shahii	100	0
GCA_0045	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
58275.1	istipes;s__Alistipes shahii	99.52	0
GCA_0004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
33435.1	istipes;s__Alistipes shahii	93.11	0.16
GCF_0058	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
44225.1	istipes;s__Alistipes shahii	100	0.48
GCA_9023	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Al		
62205.1	istipes;s__Alistipes shahii	100	0
GCA_9025	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
01405.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCA_9007	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
62265.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75845.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCA_0148	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
48045.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	97.47	6.45
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75895.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_9012	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
12465.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75835.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_9025	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
01595.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCA_9051	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
92875.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	1.61
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75925.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_9025	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
01535.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_9025	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
01435.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCF_9012	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
12495.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
41755.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	99.19	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75915.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	0.81
GCA_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75955.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_H	100	6.09
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
65115.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
49965.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68055.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
67075.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	99.19	0.81

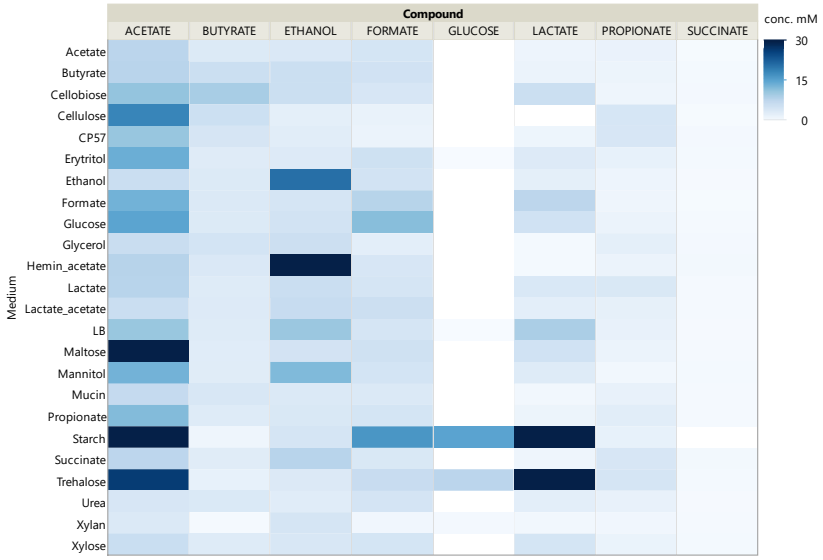
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76555.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
57485.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
67145.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	94.76	2.42
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68535.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	1.61
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
15235.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	99.19	0.81
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68585.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	92.51	7.26
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
04465.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
70025.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	99.19	3.63
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68795.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	88.68	2.76
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
60765.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
41075.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_M	100	0.81
GCF_9023	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
64945.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	0.81
GCF_0034	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
75205.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	1.61
GCF_9023	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
64355.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	1.61
GCA_9051	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
97595.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	88.16	1.61
GCF_0034	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68125.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	0.81
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
14815.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	1.61
GCA_9024	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
00055.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	0.81
GCF_0034	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
36395.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_O	100	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
42345.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	98.39	0.81
GCA_0019	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
15225.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	88.03	0.81
GCF_9023	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
63035.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	100	0.81
GCF_0014	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
06575.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
56945.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	100	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
65375.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	100	0
GCF_0014	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
05375.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	100	0.81
GCA_0148	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
71685.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_F	97.04	1.21
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
46965.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_J	95.39	1.61
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
69145.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_J	98.39	0.81
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
71135.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_J	100	0.81
GCA_0183	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76265.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_J	99.19	0.81
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
70995.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_J	99.19	0.81
GCA_0148	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
71645.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_J	99.19	0.81
GCA_9005	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
42415.1	__Ruminococcus_E;s__Ruminococcus_E bromii	99.33	1.01
GCF_9023	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
87655.1	__Ruminococcus_E;s__Ruminococcus_E bromii	99.33	1.01

GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
63965.1	__Ruminococcus_E;s__Ruminococcus_E bromii	100	1.01
GCA_0148	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
64025.1	__Ruminococcus_E;s__Ruminococcus_E bromii	97.65	0.34
GCF_0028	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
34225.1	__Ruminococcus_E;s__Ruminococcus_E bromii	99.33	1.01
GCA_9007	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
52575.1	__Ruminococcus_E;s__Ruminococcus_E bromii	96.31	0.67
GCA_9005	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
52505.1	Prevotella;s__Prevotella lascolaii	74.65	0.37
GCA_9052	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
05205.1	Prevotella;s__Prevotella lascolaii	97.21	1.11
GCF_9000	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
79775.1	Prevotella;s__Prevotella lascolaii	99.26	0
GCA_0004	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
31935.1	Prevotella;s__Prevotella lascolaii	96.65	0.31
GCA_0019	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__		
15685.1	Prevotella;s__Prevotella lascolaii	99.2	0.74
GCA_9007	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
56565.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_K	99.19	0.81
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
67915.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_K	95.16	1.61
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68235.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_K	96.81	1.61
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
68315.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_K	97.85	2.42
GCA_9051	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
97155.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_K	87.76	2.69
GCA_9003	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
15475.1	__Ruminococcus_E;s__Ruminococcus_E bromii_A	99.33	0.67
GCF_9001	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
01355.1	__Ruminococcus_E;s__Ruminococcus_E bromii_A	99.33	1.01
GCA_9027	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
69425.1	__Ruminococcus_E;s__Ruminococcus_E bromii_A	99.33	0
GCA_0035	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
18665.1	__Ruminococcus_E;s__Ruminococcus_E bromii_A	73.75	4.08
GCA_0022	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Oscillospirales;f__Acutalibacteraceae;g		
99305.1	__Ruminococcus_E;s__Ruminococcus_E bromii_A	91.99	2.75
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
11705.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_I	99.19	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
45855.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_I	93.78	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
46725.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_I	98.39	2.08
GCF_0058	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
45035.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_I	100	0.81
GCF_9023	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
74195.1	riaceae;g__Collinsella;s__Collinsella aerofaciens	99.6	1.61
GCF_0105	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
09075.1	riaceae;g__Collinsella;s__Collinsella aerofaciens	100	0.81
GCF_0001	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
69035.1	riaceae;g__Collinsella;s__Collinsella aerofaciens	99.6	1.61
GCF_9004	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
61335.1	riaceae;g__Collinsella;s__Collinsella aerofaciens	100	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
42895.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_Q	93.55	0
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
11575.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_Q	77.83	4.87
GCF_0156	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
70795.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_Q	100	0.81
GCF_0156	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
67745.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_P	98.66	2.88
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
58785.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_P	99.19	0.81
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
40965.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_P	100	0.81
GCA_0086	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
80965.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_L	100	2.42
GCA_9005	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
55775.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_L	59.68	0

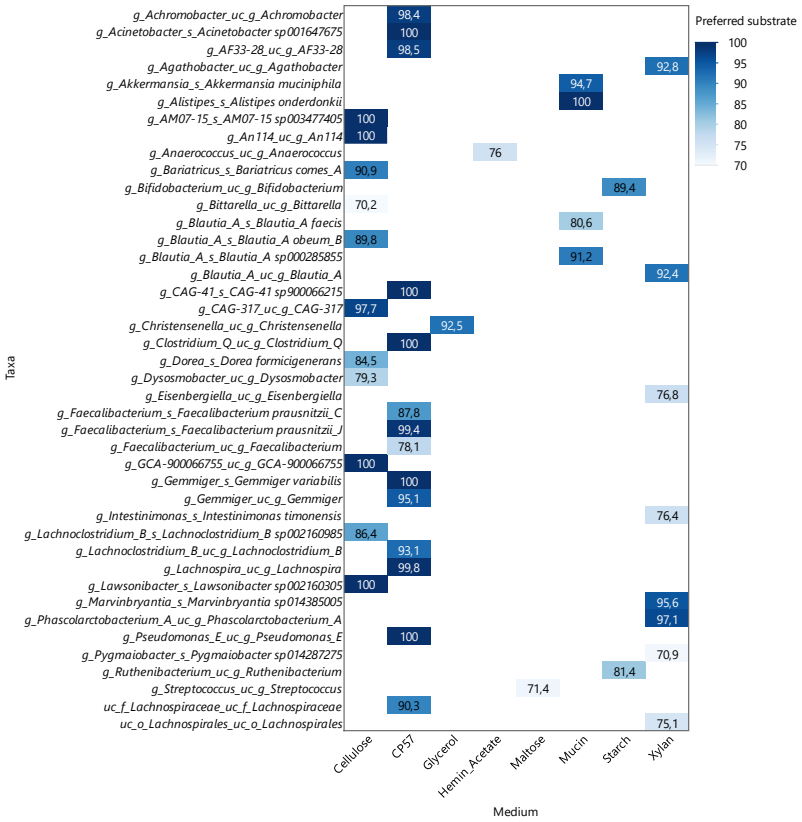
GCF_0187	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
85265.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_T	100	0.81
GCF_0156	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
70745.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_T	100	0.81
GCA_0148	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
71615.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_V	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
53355.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_V	100	1.61
GCF_0014	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
04695.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_E	99.19	0.81
GCF_9023	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
62815.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_E	99.19	0.81
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
13725.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_R	96.39	1.21
GCA_9052	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
08475.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_R	100	1.69
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76115.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_N	100	0.81
GCF_0098	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
76165.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_N	100	0.81
GCF_9023	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
88175.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_A	100	0.81
GCF_0027	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
36145.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_A	100	0.81
GCF_0155	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
49285.1	_Bariatricus;s__Bariatricus comes_A	99.42	0.49
GCF_0133	d__Bacteria;p__Firmicutes_A;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g_		
00725.1	_Bariatricus;s__Bariatricus comes_A	99.42	0.49
GCF_0191	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
31975.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_S	100	1.61
GCF_0191	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
31995.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_S	100	1.61
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
52075.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_Y	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
54145.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_AA	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
61195.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_AC	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
57435.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_Z	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
55045.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_W	100	0.81
GCF_0187	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
85245.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_AD	100	1.61
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
46965.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_X	100	0.81
GCF_0155	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
48375.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_AB	100	1.61
GCA_0148	d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacte		
46205.1	riaceae;g__Collinsella;s__Collinsella aerofaciens_AE	50.86	3.45

Supplementary Table S4.4. Pairwise interaction matrix of growth (cell count in log_{10}) compared to the monocultures.

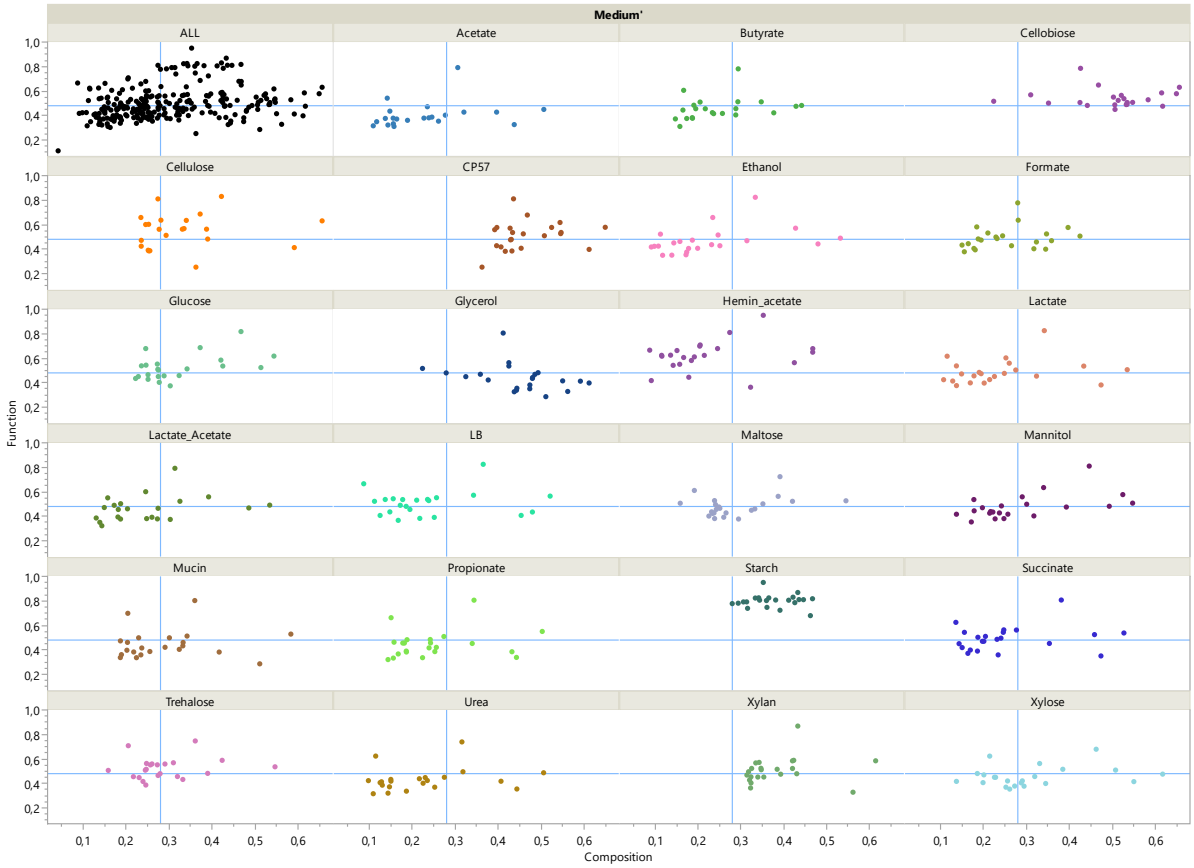
Strain	A. hadrus	A. rectale	A. shahii	B. obeum	B. vulgatu	C. aerofac	C. comes	D. longica	F. prausni	O. splanch	Prevotella	Prevotella	R. bromii
A. hadrus		0.759867	-0.00447	0.405621	0.853043	0.916874	0.813079	0.666856	0.611475	0.05975	-7.08	0.501193	-0.01562
A. rectale	-0.6944		0.052515	0.294759	0.067127	0.036141	-7.8	-7.8	-7.8	-7.8	-0.34295	-7.8	-0.34037
A. shahii	0.001972	-0.24302		0.153598	0.075371	0.39276	-8.04	0.475443	-8.04	-8.04	-8.04	-8.04	-0.3966
B. obeum	0.096441	-0.98822	-0.44774		-8	-8	-8	-8	-0.37683	-0.5016	-8	0.54909	-8
B. vulgatus	0.115476	-1.1988	0.167575	-7.88		0.239928	-2.65674	-0.43557	-0.08027	0.260668	0.137346	-7.88	-0.22475
C. aerofaciens	0.662844	0.406547	-0.11647	0.644389	0.038243		-0.49691	0.429814	-0.02887	0.522093	0.623868	0.177288	0.648045
C. comes	-0.27212	-7.7	0.107617	-7.7	0.142005	-0.17772		-7.7	-7.7	-7.7	0.392099	-1.28945	-7.7
D. longicatena	0.231355	0.588111	-7.7	-7.7	0.027752	-0.04269	-0.15931		0.358947	-7.7	-7.7	-7.7	0.454174
F. prausnitzii	-0.0059	-0.09464	-0.27737	0.402547	-7.9	0.753169	-7.9	-7.9		-1.11062	-7.9	-0.48151	-7.9
O. splanchnicus	-0.35597	-0.04206	-1.45382	-7.7	1.082295	0.29046	-7.7	-7.7	-0.54957		-7.7	-0.4258	-7.7
Prevotella copri	-8.5	-8.5	-0.61448	-1.04541	-0.74144	-8.5	-0.70152	-8.5	-1.72937	-0.19899		-1.04786	-1.77223
Prevotella sp.	-0.25867	0.98311	-7.8	0.550506	-7.8	0.578795	1.144133	-0.19286	0.4301	0.481722	0.751209		0.75294
R. bromii	-0.19905	-7.7	-7.7	-7.7	-0.11552	-7.7	-7.7	0.094526	-7.7	-7.7	-7.7	-0.00154	



Supplementary Figure S5.1: Average metabolic composition (mM) of each medium per compound

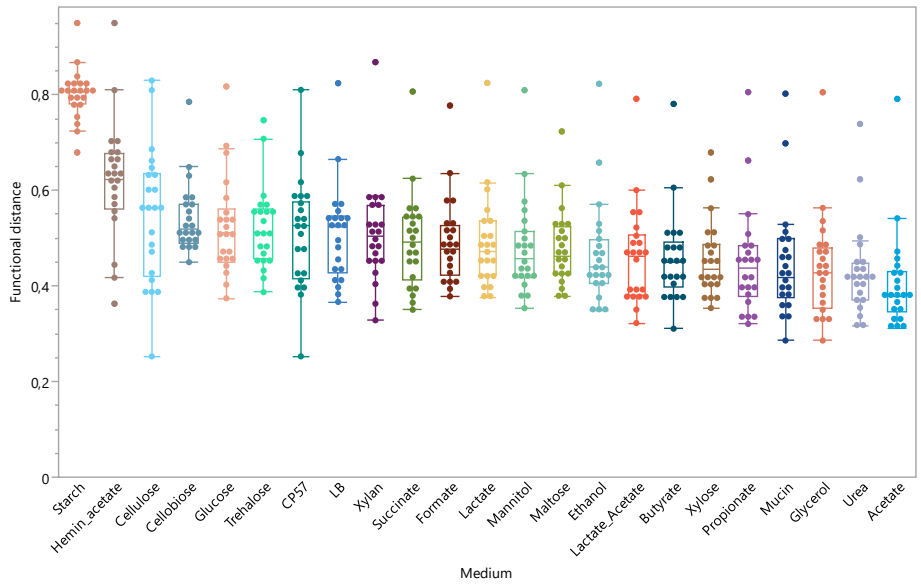


Suplementary Figure S5.2: Percentage of the total abundance found in all media per species per medium, 70% and above are shown.

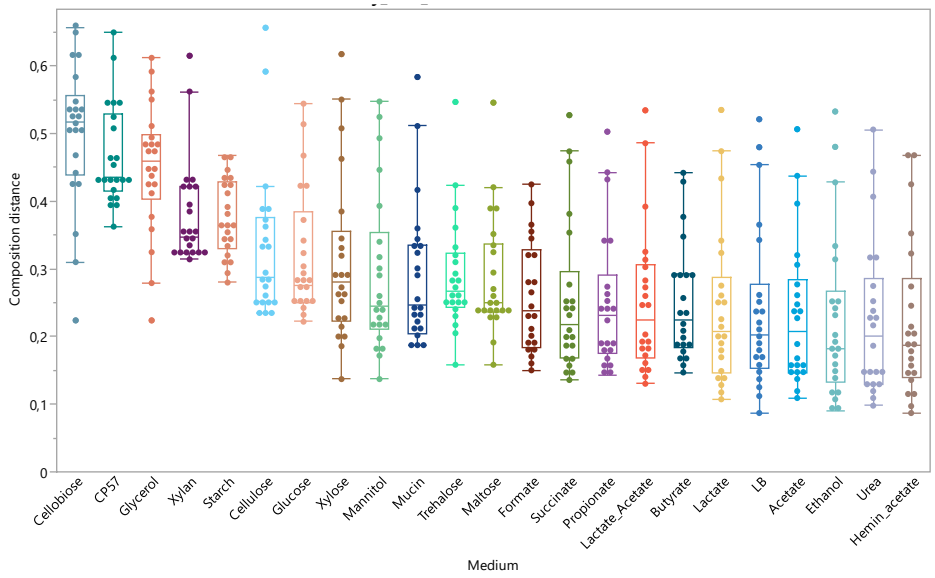


Supplementary Figure S5.3: Metabolic production distances (Euclidean distance) and compositional distance (Bray-Curtis dissimilarity) averaged between each medium split per medium, the blue lines represent the median Eu and BC distances.

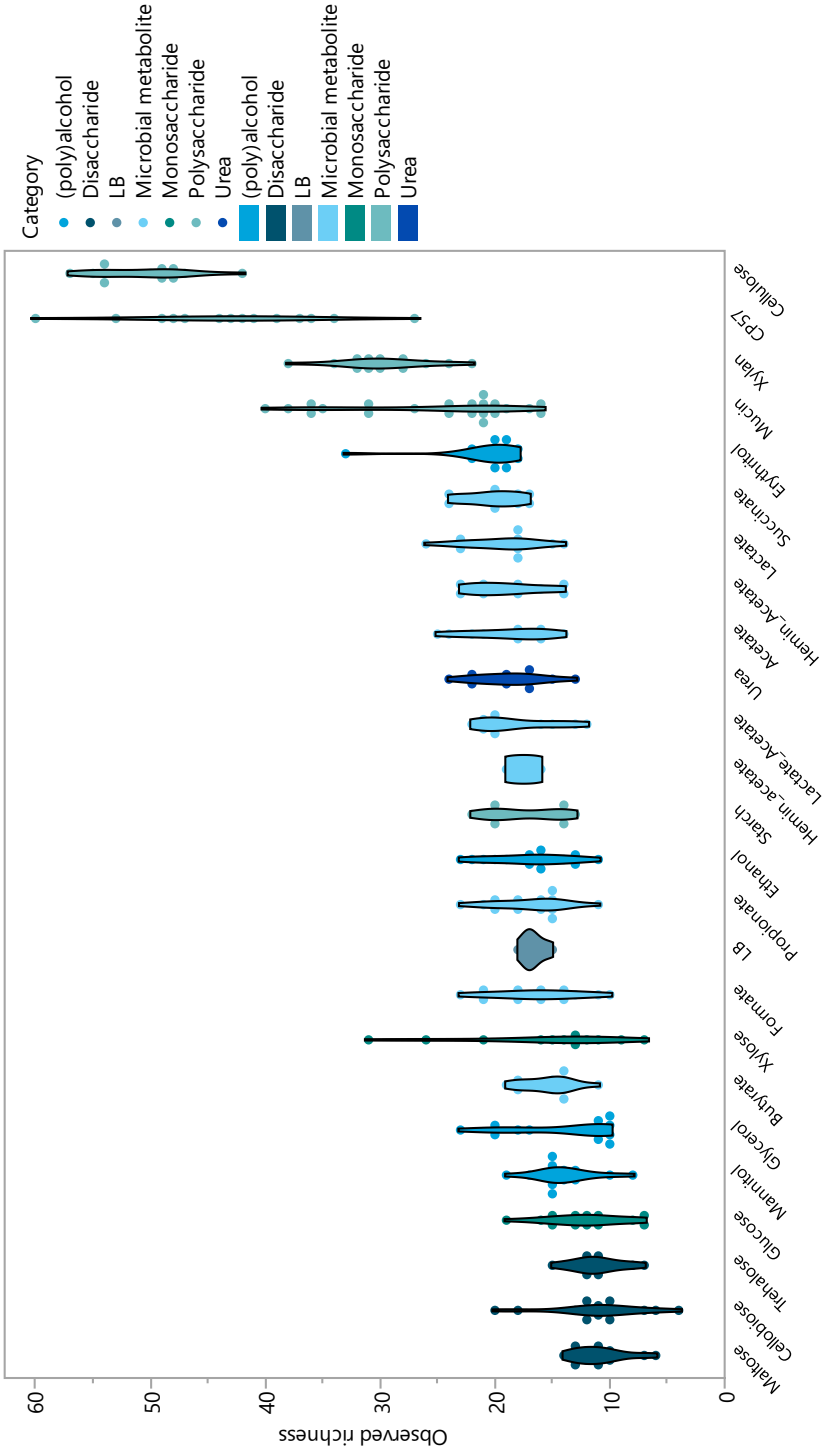
A.



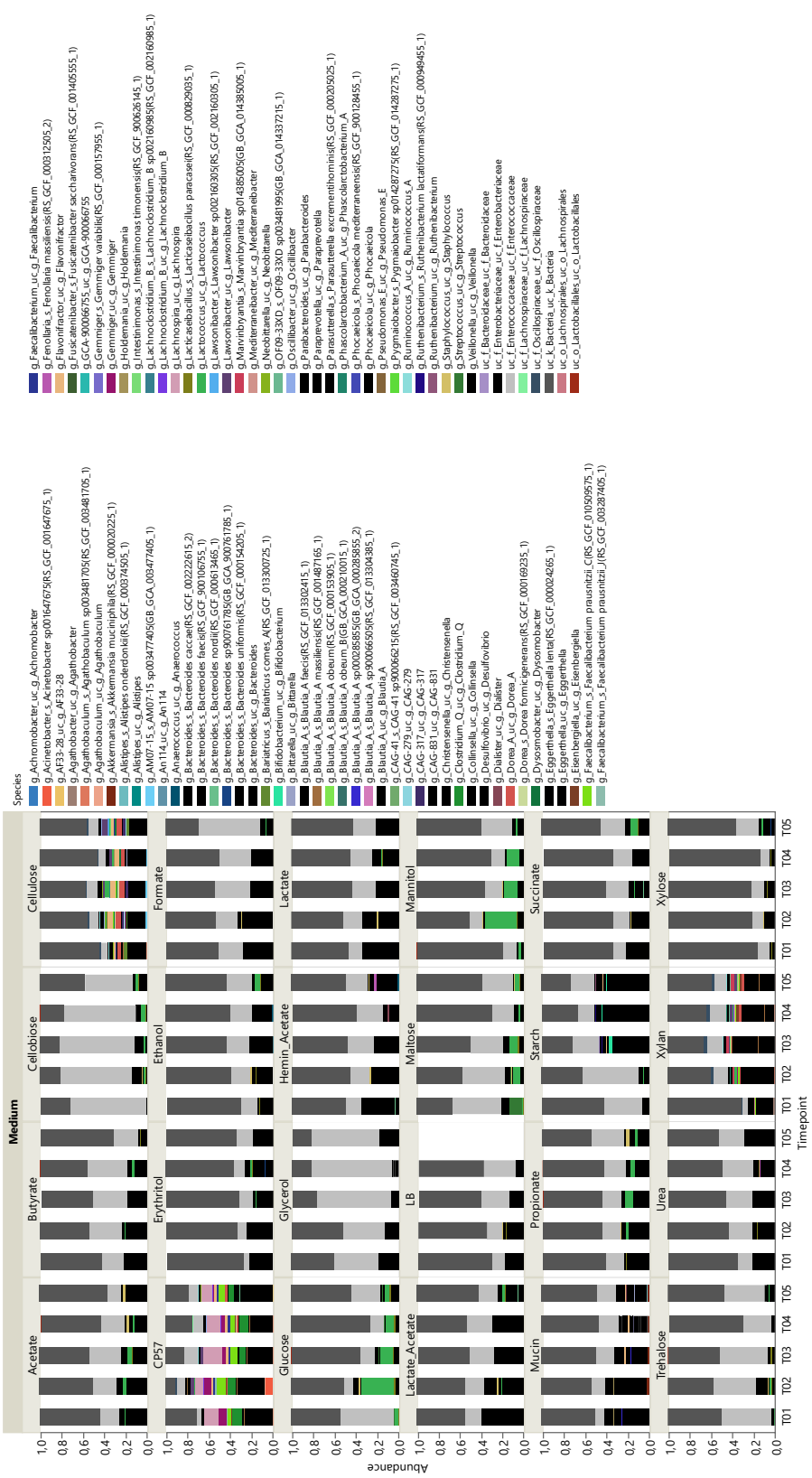
B.



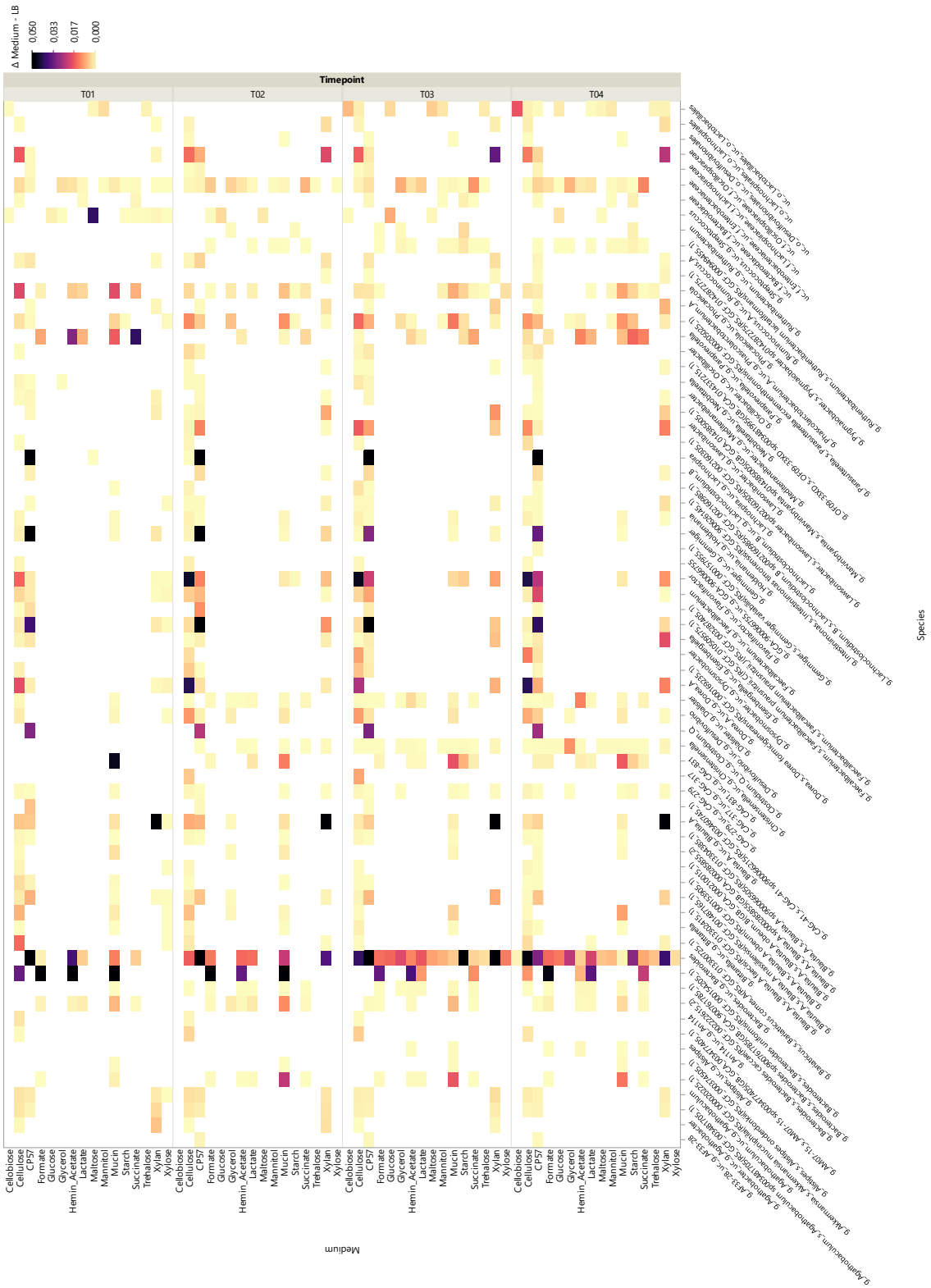
Supplementary Figure S5.4: A. Metabolic production distances (Euclidean distance) and B. compositional distance (Bray-Curtis dissimilarity) per medium ordered from high to low.



Supplementary Figure S5.5: Richness at species level per medium ordered from low to high



Supplementary Figure S5.6: Microbial composition at species level overtime per medium. In black, species found in LB, in light grey *Enterococcus* and dark grey *E.coli* blooms.



Supplementary Figure S5.7. Heatmap of the difference in abundance of each taxon in each medium compared to LB overtime.

Supplementary material chapter 6

Supplementary Table S6.1: Genera present in the inocula and fermentations for each enterotype. In red the common genera between inoculum and fermentation.

Inocula Bact1	Fermentation Bact1	Inocula Prev	Fermentation Prev
g_Phocaeicola	uc_k_Bacteria	g_Prevotella	uc_k_Bacteria
g_Agathobacter	uc_f_Enterococcaceae	g_Faecalibacterium	uc_f_Enterococcaceae
g_Faecalibacterium	g_Phocaeicola	g_Agathobacter	g_Phocaeicola
uc_f_CAG-508	g_Faecalibacterium	g_Blautia_A	g_Faecalibacterium
g_Bacteroides	g_Blautia_A	g_Holdemanella	g_Blautia_A
g_Blautia_A	g_Collinsella	g_Gemmiger	g_Collinsella
uc_k_Bacteria	g_Flavonifractor	g_Butyrivibrio_A	g_Flavonifractor
g_Ruminococcus_D	g_Parabacteroides	uc_f_Lachnospiraceae	g_Parabacteroides
g_Anaerostipes	g_Gemmiger	g_Pseudoruminococcus	g_Gemmiger
g_Roseburia	g_Akkermansia	g_UBA1436	g_Akkermansia
g_Alistipes	g_Paraprevotella	g_CAG-127	g_Bifidobacterium
g_Lachnospira	g_Bifidobacterium	g_Mediterraneibacter	g_Fusicatenibacter
g_Gemmiger	g_Fusicatenibacter	g_Phascolarctobacterium_A	g_Finegoldia
uc_f_Lachnospiraceae	g_CAG-831	g_Lachnospira	g_CAG-81
g_Pseudoruminococcus	g_Dorea_A	g_Dorea_A	g_CAG-831
g_Coprococcus	g_Eggerthella	g_Zag1	g_Eggerthella
g_Anaerobutyricum	g_Bacteroides	g_Mitsuokella	g_Bacteroides
uc_f_Bacteroidaceae	g_Parasutterella	uc_f_CAG-508	g_Parasutterella
g_CAG-279	g_Mediterraneibacter	g_Phocaeicola	g_Mediterraneibacter
g_Ruminococcus_C	g_Bariatricus	g_Flavonifractor	g_Senegalimassilia
g_CAG-196	g_Dorea	g_Anaerobutyricum	g_Bariatricus
g_Agathobaculum	g_Agathobacter	g_Ruminococcus_C	g_Dorea
g_CAG-354	g_Veillonella	g_CAG-831	g_Agathobacter
g_CAG-41	g_Lacticaseibacillus	g_Dorea	g_Veillonella
g_CAG-127	g_Lactococcus	g_Fusicatenibacter	g_Lactococcus
g_Fusicatenibacter	g_Lachnospira	g_Bariatricus	g_Lachnospira
g_Parabacteroides	g_Streptococcus	g_UMGS1375	g_Streptococcus
g_HGM11604	g_Clostridium_Q	g_Clostridium_Q	g_Prevotella
g_Parasutterella	g_Desulfovibrio	g_Parabacteroides	g_Holdemanella
g_Eisenbergiella	g_ER4	g_CAG-110	g_Peptoniphilus_A
g_Eubacterium_R	g_Agathobaculum	g_Eubacterium_G	g_ER4
g_Paraprevotella	g_Ruminococcus_A	g_ER4	g_Agathobaculum
g_CAG-110	uc_f_Enterobacteriaceae	g_Sutterella	g_Eubacterium_I
g_TF01-11	g_Lachnoclostridium_B	g_TF01-11	g_Intestinimonas
g_Bifidobacterium	g_CAG-279	uc_c_Clostridia	g_Ruminococcus_A
uc_f_Enterococcaceae	g_Dialister	uc_f_CAG-74	g_Coprococcus_A
uc_o_Bacteroidales	g_Lentilactobacillus	g_Agathobaculum	g_UBA1417
g_Mediterraneibacter	uc_f_Oscillospiraceae	g_RC9	uc_f_Enterobacteriaceae
g_CAG-83	g_Dysosmobacter	g_Anaerostipes	g_Butyricimonas
g_CAG-831	g_Staphylococcus	g_Faecalibacillus	g_Eubacterium
g_ER4	g_Phascolarctobacterium_A	g_Eubacterium_R	g_Catenibacterium
g_Desulfovibrio	g_UBA1191	g_CAG-41	g_Dialister
g_CAG-45	g_Eisenbergiella	g_Streptococcus	uc_f_Oscillospiraceae
g_CAG-81	uc_o_Lachnospirales	g_CAG-83	uc_f_Lachnospiraceae
g_CAG-1427	g_Lawsonibacter	g_Eubacterium_I	g_Dysosmobacter
g_Akkermansia	g_Oscillibacter	g_Coprococcus_A	g_Mitsuokella
g_Methanobrevibacter_A	g_Neobittarella	g_UMGS1815	g_Phascolarctobacterium_A
g_CAG-317	g_Bittarella	g_Clostridium	g_Mailhella
g_Streptococcus	g_Fenollaria	g_Ruminiclostridium_E	g_Sutterella
g_Phascolarctobacterium_A	uc_c_Bacilli	g_51-20	g_UBA1191
uc_f_CAG-74	g_Acinetobacter	g_Acetatifactor	g_Eisenbergiella
g_Faecalibacillus	g_Micrococcus	g_Adlercreutzia	g_Lawsonibacter
g_CAG-115	g_Pseudomonas_E	g_Akkermansia	g_Oscillibacter
g_UBA1417	g_Achromobacter	g_Alistipes	g_Slackia_A
g_CAG-302		g_Anaerotignum	g_Hungatella_A
g_Eubacterium_F		g_Bacteroides	g_Neobittarella
g_UBA9502		g_Bacteroides_F	g_Bittarella
g_Clostridium_Q		g_Barnesiella	g_Fenollaria
g_Enterocloster		g_Bifidobacterium	g_Duodenibacillus
g_Hafnia		g_Butyricicoccus	g_Allisonella
g_Coprococcus_A		g_CAG-115	g_UMGS1375
g_Eubacterium_G		g_CAG-1427	g_Olsenella_E
g_51-20		g_CAG-177	g_Turicimonas
g_Bariatricus		g_CAG-196	g_UC5-1-2E3
g_Flavonifractor		g_CAG-279	g_Holdemania
g_Lachnoclostridium_B		g_CAG-302	g_GCA-900066755
uc_f_Oscillospiraceae		g_CAG-317	g_Christensenella
g_Acetatifactor		g_CAG-354	g_CAG-317
g_Adlercreutzia		g_CAG-45	uc_o_Bacteroidales
g_Anaerotignum		g_CAG-81	uc_f_Ruminococcaceae
g_Bacteroides_F		g_Collinsella	g_Micrococcus
g_Barnesiella		g_Coprococcus	
g_Butyricicoccus		g_Desulfovibrio	
g_Butyrivibrio_A		g_Dialister	
g_CAG-177		g_Duodenibacillus	
g_Clostridium		g_Dysosmobacter	
g_Collinsella		g_Eisenbergiella	
g_Dialister		g_Enterocloster	
g_Dorea		g_Eubacterium_F	
g_Dorea_A		g_Evtepia	
g_Duodenibacillus		g_Fournierella	
g_Dysosmobacter		g_GCA-900066135	
g_Eubacterium_I		g_Haemophilus_D	
g_Evtepia		g_Hafnia	
g_Fournierella		g_HGM11604	
g_GCA-900066135		g_Lachnoclostridium_B	
g_Haemophilus_D		g_Lawsonibacter	
g_Holdemanella		g_Marvinbryantia	
g_Lawsonibacter		g_Methanobrevibacter_A	
g_Marvinbryantia		g_Paraprevotella	
g_Mitsuokella		g_Parasutterella	
g_Phascolarctobacterium		g_Phascolarctobacterium	
g_Prevotella		g_Romboutsia	
g_RC9		g_Roseburia	
g_Romboutsia		g_Ruminococcus	
g_Ruminiclostridium_E		g_Ruminococcus_A	
g_Ruminococcus		g_Ruminococcus_D	
g_Ruminococcus_A		g_Sellimonas	
g_Sellimonas		g_Tidjanibacter	
g_Sutterella		g_UBA1417	
g_Tidjanibacter		g_UBA1822	
g_UBA1436		g_UBA3402	
g_UBA1822		g_UBA7160	
g_UBA3402		g_UBA7173	
g_UBA7160		g_UBA7182	
g_UBA7173		g_UBA9502	
g_UBA7182		g_UMGS1994	
g_UMGS1375		uc_f_Bacteroidaceae	
g_UMGS1815		uc_f_Eggerthellaceae	
g_UMGS1994		uc_f_Enterococcaceae	
g_Zag1		uc_f_Oscillospiraceae	
uc_c_Clostridia		uc_k_Bacteria	
uc_f_Eggerthellaceae		uc_o_Bacteroidales	
uc_o_Oscillospirales		uc_o_Oscillospirales	

Supplementary material chapter 7

Materials and methods

Growth rates (Figure 1)
Strains were grown anaerobically at 37°C in mGAM (HyServe). From an overnight culture, 10 µL was transferred to into a non-treated 96-well plate containing 190 µL of mGAM medium in each well. The inoculated plate was then transferred to a BioTek Epoch II plate reader set to 37°C with linear shaking for 2 minutes before measuring the OD and programmed to make OD_{600} readings every 2 hours for 30 hours.

Ratios (Figure 2)
Two communities of 13 strains from the RCC collection were assembled in triplicates with either a 1:1 ratio or a ratio based on their relative abundance in the 16S rRNA gene sequencing dataset of the FGFP cohort. Samples were taken for cell count and 16S rRNA gene sequencing after 16, 24, 32 and 40 hours. Species richness was calculated to be compared between the two ratios.

16S rRNA gene sequencing of inoculates (Figure 3B)
DNA extraction and sequencing pipeline was performed as described in Chapter 2. In this experiment, inoculates composed of the four species from Chapter 4 assembled in a 1:1 ratio were also sent for sequencing.

MiSeq vs ONT (Figure 4A and B)
The DNA of strains used in Chapter 4 was extracted as described in Chapter 4 for communities and then used to prepare 36 pairwise species combinations with equal ratio. Subsequently, these mixes were either subjected to the 16S rRNA gene sequencing process (described in Chapter 2) or the ONT sequencing process (described in Chapter 4). The resulting compositions from the pairwise strain combinations were calculated and compared to the expected proportion (i.e., 1:1) as visualised in the heatmap as ratios Proportion/Expected (Figure 4A).
The Bact1-like M-HAEFC (Mix 1) and Prev-like M-HAEFC (Mix5) from Chapter 4 were assembled in a 1:1 ratio and subjected to sequencing using either the 16S rRNA sequencing process or the ONT sequencing process. The resulting community composition is shown in Figure 4B.

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Acknowledgement, Personal contributions, and Conflict of Interest Statements

Personal contributions

Chapter 1:
The general introduction and objectives were written by Anaïs Biclot and critically revised by Geert Huys and Jeroen Raes.

Chapter 2:
Anaïs Biclot designed the study and experiments together with Geert Huys and Jeroen Raes. Sampling of the cohort was set up and carried out by Doris Vandeputte, Kevin D'hoe, Raul Tito and Gwen Falony, as well as all faecal sample measurements. Cultivation experiments were carried out by Anaïs Biclot. Sequencing data pre-processing was done by Raul Tito and Anaïs Biclot. Sequencing of isolates and analysis was performed by Anaïs Biclot and Rodrigo Bacigalupe. Statistical analyses by Anaïs Biclot and Rodrigo Bacigalupe. The chapter was drafted by Anaïs Biclot, Geert Huys and Jeroen Raes. All authors revised the article and approved the final version for publication.

Chapter 3:
Anaïs Biclot designed the study together with Geert Huys and Jeroen Raes. Sequencing data pre-processing was done by Raul Tito and Anaïs Biclot. In silico selection of strains from the dataset and in silico metabolic analysis were done by Anaïs Biclot. Statistical analyses were done by Anaïs Biclot The chapter was drafted by Anaïs Biclot and critically revised by Geert Huys and Jeroen Raes.

Chapter 4:
Anaïs Biclot designed the study and experiments together with Geert Huys and Jeroen Raes. Cultivation experiments were set up by Anaïs Biclot and Geert Huys and carried out by Anaïs Biclot. Sequencing data pre-processing was done by Joshua Simcock. Flow cytometry data was acquired by Anaïs Biclot. Statistical analyses were done by Anaïs Biclot. Metabolic readouts were acquired by Kenneth Simoens and analysed by Anaïs Biclot. The chapter was drafted by Anaïs Biclot and critically revised by Geert Huys and Jeroen Raes.

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Vrancken and Gwen Falony and carried out by Anaïs Biclot and Jolien Van Riet. Sequencing data pre-processing was done by Raul Tito and Anaïs Biclot. Statistical analyses were done by Anaïs Biclot. Metabolic readouts were acquired by Kenneth Simoens and analysed by Anaïs Biclot. The chapter was drafted by Anaïs Biclot and critically revised by Jeroen Raes.

Chapter 7:
The general discussion was drafted by Anaïs Biclot and critically revised by Geert Huys and Jeroen Raes.

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Conflict of interest statement

Jeroen Raes is an advisor for MRM health. The other authors declare that they have no competing interests.

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Charlotte van de Velde*, Clémence Joseph*, Anaïs Biclot, Geert R.B. Huys, Vitor Pinheiro, Kristel Bernaerts, Jeroen Raes, Karoline Faust. Fast quantification of gut bacterial species in cocultures using flow cytometry and supervised classification. ISME COMMUN. 2, 40 (2022)

Jorge F. Vázquez-Castellanos*, Anaïs Biclot*, Gino Vrancken, Geert R.B Huys, Jeroen Raes Design of synthetic microbial consortia for gut microbiota modulation. Curr Opin Pharmacol. 2019 Dec; 49:52-59. doi: 10.1016/j.coph.2019.07.005. Epub 2019 Aug 17. PMID: 31430629.
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