Endogenous Small Intestinal Microbiome Determinants of Transient Colonization Efficiency by Bacteria from Fermented Dairy Products; A Randomized Controlled Trial

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Abstract

Background

The effects of fermented food consumption on the small intestine microbiome and its role on host homeostasis are largely uncharacterized as our knowledge on intestinal microbiota relies mainly on faecal samples analysis. We investigated changes in the small intestinal microbial composition and functionality, short chain fatty acid (SCFA) profiles, and on the gastro-intestinal (GI) permeability in ileostomy subjects upon the consumption of fermented milk products.

Results

We report the results from a randomized, cross-over, explorative study where 16ileostomy subjects underwent 3, 2-week interventions periodsin which they daily consumed either milk fermented by *Lacticaseibacillus rhamnosus* CNCM I-3690, or milk fermented by *Streptococcus thermophilus* CNCM I-1630 and *Lactobacillus delbrueckii* subsp. *bulgaricus* CNCM I-1519, or a chemically acidified milk (placebo). Weperformed metataxonomic, metatranscriptomic analysis and SCFA profiling of ileostomy effluents as well as a sugar permeability test andto investigate the microbiome impact of these interventions and their potential effect on mucosal barrier function. Consumption of the intervention products significantly impacted the small intestinal microbiome composition and functionality but did not affect the SCFA levels in ileostoma effluent, or the gastro-intestinal permeability. Theimpact on microbiome composition was highly personalized,andwe identified the poorly characterized bacterial family, *Peptostreptococcaceae*, to be positively associated with low abundance of the ingested bacteria. Activity profiling of the microbiota revealed that carbon- versus amino acid-derived energy metabolism of the endogenous microbiome could be responsible for the individual-specific intervention effects on the small intestine microbiome composition and function.

Conclusions

The ingested bacteria are the main drivers of the intervention effect on the small intestinal microbiota composition. Their transient abundance level is highly personalized and influenced by the energy metabolism of the ecosystem that is reflected by its microbial composition (http://www.clinicaltrials.gov, ID NCT NCT02920294).

Introduction

The human body hosts a multitude of microbial communities that occupy different body niches forming complex host-microbial ecosystems. There is increasing evidence of the importance of these microbial communities for host health and homeostasis[1, 2], with a particular focus on the colon microbiome[3]. However, the small intestine (SI) is not only the most prominent region for nutrient digestion and absorption[4] but also the intestinal region where food, bacteria and mucosa are in relatively close contact. This creates an environment of prominent microbiota interactions with the diet and the host

mucosa[5] that can be expected to be very important for human health[6]. This is exemplified by recent rodent studies that established prominent roles of the SI microbiota in whole body glucose homeostasis[7, 8], lipid digestion and absorption[9], and bile acid metabolism[10]. Moreover, the SI microbiota produces several important micronutrients like vitamin K and B12[11, 12], as well as other metabolites, such as short chain fatty acids (SCFA) that can be perceived and processed by humans and thereby affect human health.

Contrary to the colonic microbiota, the human SI microbiota is poorly characterized, due to the invasive sampling technologies required to obtain material from the SI tract in healthy subjects.This can in part be overcome by sampling from individuals who underwentcolectomy to remedy diseases like colorectal cancer, ulcerative colitis, or Crohn's disease. In some of these surgical interventions the ileum is connected to a stoma in their abdominal wall (ileostoma), allowing non-invasive sampling from the SI tract. Despite their history of intestinal disease, and provided that comorbidities are absent, these ileostomists do not need maintenance medicationand their SIis considered to function similarly as that of a healthy individual[13]. This is supportedby the similarmicrobiota composition encountered in ileostoma effluent samples compared to that in SI samples obtained from healthy subjects[14, 15].

The SI is a harsh environment for bacteria due to high concentrations of digestive enzymes, bile salts, and antimicrobial peptides. Microbial density and diversity are relatively low[15, 16], ranging from approximately 10^{4-5} to 10^{7-9} cells per mL intestinal content in the duodenum and distal ileum, respectively[16]. The SI microbiota is predominantly composed offacultative anaerobic bacterial taxa[17], displaying higher compositional dynamics compared to the colon due to rapid responses to the changing nutrient availability[14]. Metagenomic and metatranscriptomic studies corroborated the dynamic nature of the SI microbiota that comprises a broad repertoire of catabolic pathways for simple sugars, rather than complex carbohydrates that are enriched in the colonic microbiota[14, 15].These findings have started to shed light on the human small intestinal microbiome and pave the way towards a better understanding of its role in health and disease.

Several food products are rich in viable bacteria that may transiently dominate the SI ecosystem. For example, yogurt is a commonly consumed fermented dairy product containing more than 10^9 viable bacteria between*Lactobacillus delbrueckii* subspecies *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* per mL. Likewise, probiotics that are defined as "live microorganisms that, when consumed in adequate amounts, confer a health benefit on the host"[18],are typically consumed at 10^{8-12}viable bacteria per serving. Nevertheless, only few studies have investigated the effect of live bacteria ingestion on the small intestinal microbial population composition and function[19-21]. Probiotics have been shown to deliver beneficial effects on gut functions in randomised controlled studies[22], and commonly contain strains belonging to the genera*Bifidobacterium*or the bacteria formerly known as species within the genus *Lactobacillus*, which has recently been taxonomically reclassified into 23 novel genera[23]. Among the latter, *Lacticaseibacillus rhamnosus* is considered a potential probiotic, and strain-specific relief of symptoms in diseases associated with intestinal microbiota dysbiosis and maintenance of the gut homeostasis have been reported[24-26]. Specifically, preclinical studies underlined the role of *L.*

rhamnosus CNCM I-3690 in restoration of impaired intestinal barrier functionsthrough its anti-inflammatory effect[27, 28], reduction of inflammation in a murine model for colitis[29] and suppression of the immune and metabolic impairments caused by the pathobiont *Bilophila wadsworthia[30]. In addition, the strain* supported several other health benefitslike limiting weight gain, improving glucose-insulin homeostasis, and hepatic steatosis[31]. Moreover, a recent human randomised controlled trial established the safety of *L. rhamnosus* CNCM I-3690 consumption, and reported on its persistence in the human digestive track and the limited impact on faecal microbiota when combined in a multi-strain fermented milk product[32]. These preclinical findings underpin the interest in this strain as a probiotic candidate.

The aim of the present study was to investigate how fermented dairy products could (transiently) impact the small intestinal microbiota in human subjects. To this end, 16 ileostomists were recruited that underwent 3, randomised, cross-over interventions of 2 weeks, during which they consumed dairy products, fermented with *Lacticaseibacillus rhamnosus*CNCM I-3690, or yogurtthat was produced using *S. thermophilus* CNCM I-1630and *L. bulgaricus* CNCM I-1519, or a chemically-acidified milk product that served as a placebo. Ileostoma effluent samples were collected at regular intervals during these interventions and the intermittent wash-out periods (2 weeks) for microbiota composition analysis. In addition, at the start and end of each intervention period, SI microbiota activity was assessed by metatranscriptomic analysis, and SI mucosal permeability and short-chain fatty acid levels in the stoma effluent were determined.

Materials And Methods

Subjects

Adult ileostomy patients without comorbidities were recruited for this interventions study, using specific in- and exclusion criteria (see supplemental methods “recruitment criteria”). Importantly, to avoid confounding effects on the study endpoints, study subjects were not allowed to use pro-, pre- or symbiotics during the study period and three months prior to participation in this trial (a list of forbidden products was provided).

Due to a lack of reliable prior art in the investigation of the impact of food-derived bacteria on the small intestinal microbiota, no effect size could be estimated or a priori power calculation could be performed. Therefore, the study was designed as an explorative study, aiming for the inclusion of 15 to 20 subjects fulfilling the inclusion criteria. We recruited 15 subjectswith a standard ileostoma, and a single subject with a continent-ileostoma (i.e., Kock pouch). During data analysis, the SI microbiota of the latter individual was drastically different from the rest of the ileostomists and was therefore considered to be a biological outlier that was excluded from further analyses (see results).

Study design

In thisrandomized, double-blinded placebo-controlled crossover study, ileostomy patients consecutively received threedifferent interventions (one serving per day) for 14 consecutive days each (+/- one day) with a two-week wash-out in between. Moreover, the study started with a two-week run-in period and ended with a two-week run-out period.At the first and last day of each intervention period (referred to as “test days” and labelled V1-V2, V3-V4 and V5-V6 for the first, second and third intervention respectively), subjects were asked to visit the testing facility (Additional file 1, Supplementary Figure 1). An external provider, AtlanStat, prior to the start of the study generated the randomization list used for the succession of product intervention (6 sequences of 3 interventions, balance on 20 subjects, block size: 6). The list was forwarded to the person responsible for preparation of study products in Danone for the purpose of labelling.

All intervention products were manufactured by Danone (Danone Nutricia Research, Palaiseau, France) with the intention to minimize their distinction on basis of appearance and taste, including the use of similar packaging and the use of the same flavour compounds, andfully blinded. Intervention products were 100 mL of milk fermented by *Lacticaseibacillus rhamnosus*CNCM I-3690 (*L. rhamnosus*) at approximately 1-5x10^9 CFU/mL (i.e., daily intake ~ 1-5x10^{11} CFU), milk fermented by the yogurt symbionts *Streptococcus thermophilus* CNCM I-1630 and *Lactobacillus delbrueckii* subsp*. bulgaricus* CNCM I-1519 (yogurt)at approximately 10^5-10^6CFU/mL for *L. bulgaricus* and5x10^7-10^9CFU/mL for *S. thermophilus* (i.e., daily intake ~10^7-$10^8$and ~ 5x10^9-10^{11} CFU, respectively), and a milk product that was chemically acidified with ortho-phosphoric acid (placebo).The viable bacteria in the products were enumerated by plating of appropriate dilutions and colony forming unit determination. Intervention products were taken orally, one serving per dayduring breakfast.

Subjects participated in an approximately 100 days long trial with 3 intervention periods(Additional file 1, Supplementary Figure 1). Subjects collectedileal effluent samples after breakfast,for metataxonomic analysis, at home or during test days in the testing facility in 15 mL collection tubes (screw cap faeces container tubes, Sarstedt, Germany) containing 2 times concentrated DNA shield (Zymo Research, CA, USA)and stored these in a portable freezer(-18 ˚C ± 4 ˚C), on days 1, 7, and 13 during the run-in period, on days 3, 6, 9, and 12 of each intervention period, on days 7 and 13 of each wash-out period and on days 7 and 14 of the run-out period.At each test day (V1-V6), a large volume (50-100 mL of ileostomy effluent) for metatranscriptome analysiswas collected during the first five hours after takinga standardized breakfast (that included the intervention product), and wasdirectly mixed with RNA-later (Sigma, Germany), stored overnight at 4°C ± 2°C, and then transferred to a freezer (-80 °C ± 4 °C).At the same sampling timepoint, a small effluent sample (15 mL; see below)was collected for SCFA profiling that was immediately stored at -80 ˚C until analysis, as detailed previously[33]. At the start and end of each intervention period gastro-intestinal (GI) permeability was assessed by a mixed sugar test (Additional file 1, Supplementary information).

Compliance to dietary interventions and to the restrictions was checked by asking whether subjects consumed the products and whether they were able to abstain from food on the forbidden food list on every test day, revealing only few minor deviations (Additional file 1, Supplementary Table 1). Gastro-

intestinal symptoms, occurrence, and severity of adverse events during and after consumption of the three products were reported and recordedduring the entire study period (Additional file 1, Supplementary Figure 1).

Intestinal Microbiota composition and functional profiling

A detailed description of the methods used for the metataxonomic and metatrascriptomic analysis is provided in Supplementary materials.

Data mining and statistics

Descriptive statistics were calculated for age, BMI and gender (Additional file 1, Supplementary Table 2). SCFA and intestinal permeability data was analysed using IBM SPSS statistics 25 (IBM Corporation, Armonk, NY, USA) and a Kolmogorov-Smirnov tests as well as a visual check of normality (QQ plot) of the data were performed. Intervention effects on SCFA and GI permeability were assessed by mixed model analysis on baseline-corrected data. A $p<.05$ was considered statistically significant. Full details of all statistical analyses are provided in theAdditional file 1, Supplementary Materials. All authors had access to the study data and reviewed and approved the final manuscript.

Results

Study population, compliance, adverse events and secondary outcomes

For this study, 18 ileostomy subjects were screened of which 16 (Additional file 1, Supplementary Table 2,additional file 4,

Supplementary CONSORT flow diagram)were enrolled. Fifteen subjects completed the protocol (see Materials and Methods).In general compliance was high and no serious adverse events were reported during this trial (Additional file 1, Supplementary Table 1, Supplementary Figure 2).

The primary aim was to determine the impact of the consumption of fermented dairy products on the small intestine microbiota composition and activity. In addition, intestinal permeability and SCFA levels in ileostoma effluent samples were determined at the start and end of each of the intervention periods. Notably, neither permeability nor SCFA amount or composition was significantly affected by any of the interventions (Additional file 1, Supplementary Figures 3, 4 and 5).

Longitudinal metataxonomic analysis of the small intestinal microbiota

Longitudinal microbial composition in ileostomy effluent was analysed by metataxonomic analysis, which was successful in 390 (>90%) of the 432 collected effluent samples (27 per individual). The samples obtained from two subjects had the lowest success rate, which for at least one of the subjects appeared to be due to the low DNA recovery in the samples (Additional file 1, Supplementary Table 3). There was no difference in the successrate in obtaining data across the different periods of the trial (Additional file 1, Supplementary Table 4). Initial principal component analysis (PCA)analysis of the microbiota data obtained (Additional file 1, Supplementary Figure 6) revealed thatall samples obtained from one subject strongly deviated from the rest. This subject was the only individual with a Kock's pouch rather than the standard ileostoma, which led us to exclude this subject in further analyses as a biological outlier. In the remaining samples, 9270 operational taxonomic units (OTUs) were identified, representing 258 genera, and 9 phyla.

The majority of the sequences were assigned to Firmicutes (78.6%) and Proteobacteria (11.9%), followed by Actinobacteria (6.3%) and Bacteroidetes (3.1%). The average microbiota composition per subjectrevealed a high degree of variation among the subjects (Supplementary Figure 6). Redundancy analysis revealed that almost half (46.6%) of the overall microbiota composition at species level was explained by inter-subject variation (Additional file 1, Supplementary Figure 6). In addition, longitudinal composition analysis per subject revealed remarkable time-dependent fluctuation during the study period, although composition appeared more stable in some individuals (Additional file 1, Supplementary Figure 6). Nevertheless, intra- and inter-subject comparison of Bray-Curtis dissimilarity during the study showed a higher degree of variance between subjects than within subject (Additional file 1, Supplementary Figure 7).These metataxonomic analyses expand insights gained in previous studies[14, 15], by confirming the high degree of variance of the SImicrobiota composition between and within volunteers, while establishing that a personal microbiota signature is recognizable despite temporal fluctuation.

Impact of fermented dairy product consumption on the SI microbiota composition

The fermented milk products consumed during the study contained either *Lacticaseibacillus rhamnosus*CNCM I-3690 or the yogurtstarter culture bacteria *Streptococcus thermophilus* CNCM I-1630 and *Lactobacillus delbrueckii* subsp*. bulgaricus* CNCM I-1519. The placebo control product was unfermented, acidified milk. In agreement with the relatively low numbers of *L. bulgaricus*(see Materials and Methods)in the yogurt product, this species remained undetected in the effluentmicrobiota following consumption. Thereby, detection of product derived bacteria (PDB) in the effluent microbiota was restricted to the abundant bacteria inthe respective products, i.e., *L. rhamnosus*, and *S. thermophilus*. Importantly, thesebacteria were not detected in any sample obtained outside of the product-specific intervention periods, showing PDB transiently inhabit the small intestinal nicheand rapidly disappearonce consumption is stopped and establishing that the two-week washout suffices to avoid microbial carry-over between intervention periods, which is also confirmed by the absence of a detectable difference between the last samples obtained during the wash-out periodand those obtained during the run-in (Additional file 1, Supplementary Figures 1,8).

In samples obtained during the intervention periods, PDB corresponding to the intervention product could be detected (Figure 1). On average, *L. rhamnosus* and *S. thermophilus* constituted 8.5, and 5.2% of the total microbiota in the effluent samples collected during the 2 intervention weeks. However, highly variable relative abundances were observed when comparing intra- and inter-personal samples. Illustratively, 3 subjects had a PDB average relative abundance below 1% (max 5%) and the PDB could not be detected (<5.7E-03) in some of the samples, whereas 3 other subjects had a PDB average abundance above 10%, including the striking maximum relative abundance of *L. rhamnosus*at 88.2% in one of the samples. These observations exemplify the strong oscillation of relative abundance of the PDB in all subjects (Figure 1), supporting the dynamic nature of the small intestinal microbial community.

Despite the large impact of the PDB in some samples, the alpha diversity of the effluent microbiota remained unchanged during the intervention periods as compared to the run-in, although alpha diversity varied substantially between the subjects (Additional file 1, Supplementary Figure 9). The impact of the intervention on the microbiota composition was analysed by subject corrected redundancy analysis (RDA), revealing a significant effect of the intervention at both genus and species level, albeit that these effects only explained a small fraction of the overall variation (Figure 1). The drivers of the significant separation of the intervention-specific samples were the PDB (Figure 1). Moreover, species-level beta diversity analysis revealed that the samples obtained during the interventions were significantly different (Additional file 1, Supplementary Figure 10), which was supported by the inclusion of the PDB in the coremicrobiome of the effluent samples during the respective intervention periods. Notably, the PDB addition to the core microbiome was the sole difference detected in this analysis (Additional file 1, Supplementary Figure 11).

Interventions significantlyaffect the SI endogenous microbiome

The predominant effect of the product interventions on the small intestine microbiota composition appears to be the presence of PDB in samples collected during the intervention period. To investigate the possible effect of the interventions on the endogenous microbial community, the PDB-related OTUs were removed followed by recalculation of the relative abundances of the remaining OTUs. The resulting dataset was used for subject corrected RDA analysis demonstrating that samples could still be significantly separated on basis of the intervention period in which they were taken (Figure 1), albeit that samples displayed a large overlapped, and only a very low amount of variation could be explained (0.44% and 0.48% at species and genus level, respectively). The 11 endogenous species associated with the different interventions were detected by empirical analysis of digital gene expression (EdgeR)differential abundance analysis, correcting for subject ID (Additional file 1, Supplementary Table 5). However, none of these species were prevalent among the majority of subjects or present at relative high abundance (max prevalence: 27%, max abundance 1.5%). These findings indicate that the intervention-impacton the endogenous microbiota was poorly conserved and barely detectable, which agrees with the minimal amount of explained variation found by RDA.

Endogenous *Peptostreptococcaceae* abundance is correlated with the relative abundance of PDB during intervention

The analyses above indicated that the average relative abundance of the PDB during the intervention periods differed substantially per subject(Figure 1). Remarkably, high congruency was observed between the subject-specific average relative abundance of *S. thermophilus* and *L. rhamnosus* during the respective interventions, suggesting PDB colonization efficiency is very subject specific but independent of the PDB species.This led us to investigate whether the endogenous baseline microbiota could explain these differences in abundance of *L. rhamnosus* and *S. thermophilus*. The corresponding RDA analysis revealed that while several bacterial families appeared to be enriched in subjects that displayed high relative abundance values for the PDB, only the *Peptostreptococcaceae* were negatively associated with the detected abundance of the PDB (Figure 2). The significant negative correlation between PDB colonization efficiency and endogenous *Peptostreptococcaceae* relative abundance could be confirmed by univariate analysis. Notably, despite substantial variation within and between subjects, we could not detect a correlation between the DNA yield obtained from ileostoma effluent samples and *Peptostreptococcaceae* or PDB average relative abundance (Additional file 1, Supplementary Figure 12), indicating that the abundances of neither *Peptostreptococcaceae*nor PDB is associated with a difference in bacterial density. Intriguingly, high *Peptostreptococcaceae* relative abundance was correlated with a lower microbiota diversity (Figure 2).

Colonization efficiency of PDBcorrelates with elevated expression of carbon fermentation pathways in the small intestine microbiota

The microbial activity in ileostomy effluent was analyzed by metatranscriptome analysis in the samples obtained on the first and last days of the intervention periods.Functional metatranscriptome mapping (FMM) of the small intestine microbiome were obtained for each sample by genome,protein and pathway mapping.

Inter subjectFMM differences were the predominant source of variation, explaining almost 25% of the overall FMM variance (Additional file 1, Supplementary Figure 13).This is supported by the higher Bray-Curtis distance between FMM data obtained for different subjects, compared to longitudinal FMM data from a single individual irrespective of the intervention period (Additional file 1, Supplementary Figure 13).Nevertheless, subject-corrected RDAanalysis revealed that the interventionsdid significantly affect the FMM and could explain 4.64% of the overall variance in the FMM data (Additional file 1, Supplementary Figure 14). The differentially expressed microbial pathways that underly the intervention-associated effect on the FMM were identified using EdgeR differential expression analysis, with subject ID as a co-variate. This revealed that only expression of the L-rhamnose degradation I pathway was significantlyincreased in the FMM associated with the *L. rhamnosus*intervention relative to the placebo intervention (Additional file 1, Supplementary Table 6), while no FMM effects were found when comparing yogurtand placebo interventions. Notably, comparative FMM analysis of samples obtained during the yogurt and *L. rhamnosus*interventions revealed the increased expression of 8 carbon- and

fermentation- associated pathways during the *L. rhamnosus* intervention, including the L-rhamnose degradation I pathway(Additional file 1, Supplementary Table 6).These results show that the consumption of the *L. rhamnosus*fermented product associates with elevated expression of carbon metabolism pathways in the small intestinal microbiota, which is especially apparent for the L-rhamnose degradation pathway.

Endogenous microbiome pathway activity patterns influencethe colonization efficiencyofPDB

To investigate whether variations in the FMM data were associated with the highly variable and subject-specific relative abundance of PDBduring the intervention periods, RDA analysis was performed using the FMM determined during the intervention periods to explain the average relative abundance of PDBduring their respective interventions(Figure 3). A strikingly strong enrichment was found for various bacterial pathways related to amino acid metabolism in samples displaying the lower PDB relative abundance, which was contrasted by an association of glycolytic pathway expression (i.e., Glycolysis IV in EcoCyc) with the higher PDB relative abundance (Figure 3). These findings were confirmed using the multivariate association with linear models (MaAsLin2) analysis, which also pointed to positive associationsof PDB abundance and glycolytic pathway activity (Glycolysis IV and Glycolysis III), while expanding this to the Stachyose degradation pathway (Figure 3). MaAsLin2also confirmed the negative association between PDB abundance and the expression of the majority (9 out of 12) of amino acid biosynthesis pathways identified in FMM (Figure 3).Subsequently, metagenomic phylogenetic analysis (MetaPhlAn) of the FMM data enabled the phylogenetic classification of these differentially expressed pathways, revealing that the activated amino acid biosynthesis pathways were assigned to a broad range of bacterial families in a scattered manner (Figure 3), encompassing most of the microbial families encountered within the small intestinal ecosystem (30 out of 41). In this context, it is striking that the family of the *Peptostreptococcaceae* is one of the few bacterial taxa that negatively correlated with the amino acid biosynthesis pathways in the FMM.Taken together, our results highlight how the endogenous microbiota composition and its predominantly active energy metabolism could explain the individual-specific PDB colonization efficiency, specifically identifying the *Peptostreptococcaceae*family as a microbial group that prominently reflects, and is likely involved in,these individualized effects.

Discussion

In this study, we present the longitudinal metataxonomic study of the small intestine microbiota, including diet intervention periods to assess the microbial impact of the consumption of bacteria-rich fermented dairy products. Although metataxonomic analyses were the focus of this study, we also determined whether the interventions affected small intestinal mucosal permeability or the SCFA composition of the stoma effluent, revealing that neither of these parameters was significantly affected during the dairy product interventions. Nevertheless, the SCFA measurements confirmed previous observations that substantial amounts of microbial fermentation end-products are already formed in the

small intestine[14], suggesting that their profound effects on mucosal and systemic functions of the host[34] is exerted throughout the length of the intestinal tract rather than predominantly in the large intestine. The permeability measurements in this study were inspired by previous studies showing that*L. rhamnosus* CNCM-I 3690 (the strain that was also used here) could restore impaired intestinal barrier functions in mice[27, 28]. The lack of confirmatory observations in the present cohort of ileostomists may be due to the fact that contrary to the studies using mouse models, the ileostomists participating in this study did not suffer from impaired mucosal barrier function,minimizing the potential of dietary interventions to elicit an improvement,illustrative of translational difficulties across different mammalian models[35].

Our metataxonomic results confirmed the previously reported high degree of difference of the small intestinal microbial composition between and within subjects[15, 36]. In the present study almost half (46.6%) of the overall gut microbiota variation was determined by the individual. Although extensive longitudinal fluctuations within an individual were detected, a personal microbiota composition signature was recognizable, analogous to what has been found in the faecal microbiome[37, 38].Metataxonomic analyses also revealed striking variation in the average relative abundance of the PDB between and within individuals. Despite this variability, the PDB were part of the coremicrobiome during the respective interventions in all subjects. Moreover, the PDB were the predominant determinants in the metataxonomic separation between the intervention periods and the run-in and intermittent wash-out periods, demonstrating the limited and/or poorly conserved impact of the consumption of fermented foods on the composition of the endogenous microbiome. These results are in agreement with an invasive endoscope perfusion study in healthy subjects that showed that *Lactocaseibacillus casei* transiently amounted up to 75% of the ileal microbiota community in only one of the four participating individuals[20]. Similarly, highly individualized colonization patterns have been reported for bacteria derived from fermented food products and probiotics in studies focussing on the faecal microbiota[39]. This led to the hypothesis that colonization-permissive and -resistant endogenous microbiome communities determine this individual-specific colonization efficacy[21]. However, the high impact of food-borne bacteria is uniquely observed in the small intestine, since studies targeting the faecal microbiome described much more subtle effects in terms of the transient PDB relative abundance[20, 40].

We expanded the metataxonomic analysis in this study with metatranscriptome analysis of the small intestine microbiota at the start and end of each of the intervention periods, using genome mapping and FMM analysis to determine the activity profile of the small intestine microbiome. Notably, comparative analysis of the metatranscriptome FMM patterns also showed that the individual from which the sample was taken was the co-variate that explained most of the observed variance in FMM (~25%). Nevertheless, the intervention period could explain approximately 5 % of the total FMM variance, identifying the activation of the L-rhamnose degradation pathway during the consumption of the *L. rhamnosus* fermented product relative to the other interventions. This likely relates to presence of this pathway in the *L. rhamnosus* genetic repertoire (data not shown) and its low expression level by the endogenous microbiota, which could favour *L. rhamnosus*colonization as the main degrader of this sugar. This analysis illustrates that metatranscriptome analyses reveals a comprehensive and individualized

functional view of the microbiome, and allows to detect functional impacts of diet intervention and/or transient PDB abundance.

The striking variation in subject-specific relative abundance of the PDB allowed a stratified interpretation of the metataxonomic data, identifying the strong association of the abundance of the *Peptostreptococcaceae* family in the endogenous microbiota and the capacity of the PDBto effectively occupy this ecosystem in a transient yet robust manner. High *Peptostreptococcaceae* abundance correlates with lower microbial diversity of the endogenous microbiota and a lower abundance of PDB, expanding the previously proposed probiotic-permissive and -resistant faecal microbiota hypothesis[21] to the small intestine and pinpointing*Peptostreptococcaceae* as anindicator of a resistant SI microbial community. Members of this family may generically limit the inhabitation of the niche by other microbial groups, resulting in lower diversity, but also suppressing transient colonization by PDB. Metatranscriptome FMM analysis demonstrated that higher relative abundance of the PDB is associated with a microbial ecosystem that executes a carbon-derived energy metabolism, whereas lower PDB abundance associates with a variety of amino acid biosynthesis pathways that are assigned to a variety of microbial families within the ecosystem, notably excluding the *Peptostreptococcaceae*. These results imply that an environment rich in carbohydrates creates a favourable niche for the temporal colonization of by both *L. rhamnosus* and *S. thermophilus*, which agrees with the notion that these bacteria derive most of their metabolic energy from (simple sugar) carbohydrate fermentation[41] In contrast, an environment poor in fermentable carbohydrate sources would direct the endogenous microbiota towards amino acid derived energy metabolism, which could lead to low availability of amino acids that elicits the activation of amino acid biosynthesis pathways in various microbes. The latter niche characteristics would restrain the colonization capacity of the PDB, which is supported by the negative association of arginine biosynthesis pathway-activity and the abundanceof the arginine auxotrophic *L. rhamnosus*(data not shown).Interestingly, amino acid exchange has been identified as a crucial driver of microbial community interaction, and amino acid cross-feeding is highly prevalent in cooperative communities[42-44]. This agrees with the *Peptostreptococcaceae*as indicators for probiotic-resistant microbial community because they are encountered in protein rich environments where amino acids serve as the predominant energy source, which can include human and animal intestinal samples[45-47]. Moreover, several *Peptostreptococcaceae* are known to ferment (sulphur-containing) amino acids[48], or have been reported to be non-saccharolytic, supporting the importance of amino acid derived energy metabolism in this family[45].

Conclusions

This study shows that the consumption of milk products fermented by *L. rhamnosus*CNCM I-3690, or the yogurt symbionts *Streptococcus thermophilus* CNCM I-1630 and *Lactobacillus delbrueckii* subsp*. bulgaricus* CNCM I-1519 results in both compositional and functional changes of the SI microbiota.These changes are highly personalized, which we propose to correspond to subject-specific composition of the endogenous SI microbiotaand its predominating carbon- versus amino acid-derived energy metabolism activity.Moreover, we pinpoint the relative abundance of the poorly characterized bacterial family of the

Peptostreptococcaceae as an indicator for these subject-specific small intestine characteristics. At present, we do not know the mechanistic foundation of these subject specific small intestinal microbiome characteristics, which could be caused by differences in diet- or behaviour-related habits. Alternatively, and analogous to what has been shown for the large intestine microbiome[49, 50]by subject-specific variations in (macro-)physiology of the intestinal tract,like variations in stomach pH, stomach emptying rate, bile and digestive enzyme production levels, or small intestine transit time. Very little is known about these parameters and there is a lack of non-invasive methodologies to accurately determine them in an individual. These potentially important variables are not considered in most diet and microbiota investigations, warrantingfurther investigation of their variation among individuals since they could profoundly affect the relationships between diet, microbiota, and human physiology and health. Thereby these parameters could be critical determinants in individualized metabolic responses to dietary ingredients[51, 52], and may provide a plausible mechanistic explanation for the relationship of the endogenous SI microbiome with the transient colonization capacity of PDB.

Declarations

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Contributions

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The author(s) read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Maastricht University (MU), Maastricht, the Netherlands, and performed in full accordance with the Declaration of Helsinki (latest amendment by the World Medic Association in Fortaleza, Brazil, in 2013) and Dutch Regulations on Medical Research involving Human Subjects (WMO, 1998), as well as with the International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) guidelines. The study was performed at MU from October 27th, 2016 (screening visit, first subject) until October 27th, 2017 (last visit last subject). This study was registered in the US National Library of Medicine (http://www.clinicaltrials.gov, ID NCT NCT02920294). All subjects gave written informed consent before screening.

Consent for publication

Not applicable.

Competing interests

This author discloses the following: T. Smokvina is an employee of Danone Research. The remaining authors disclose no conflicts.

Availability of data and materials

The datasets supporting the conclusions of this article are available in the DANS repository, on the following doi: 16S https://doi.org/10.17026/dans-xhw-dhpr, Metatranscriptome https://doi.org/10.17026/dans-xvh-yww8, Permeability test https://doi.org/10.17026/dans-xnp-nkq8, Short Chain Fatty Acid profiling https://doi.org/10.17026/dans-z3q-vvz4.

References

1. McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Loso T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF *et al*: Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci U S A* 2013, 110(9):3229-3236.
2. Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ: Role of the microbiome in human development. *Gut* 2019, 68(6):1108-1114.

3. Schmidt TSB, Raes J, Bork P: The Human Gut Microbiome: From Association to Modulation. *Cell* 2018, 172(6):1198-1215.
4. Volk N, Lacy B: Anatomy and Physiology of the Small Bowel. *Gastrointest Endosc Clin N Am* 2017, 27(1):1-13.
5. Booijink CC, Zoetendal EG, Kleerebezem M, de Vos WM: Microbial communities in the human small intestine: coupling diversity to metagenomics. *Future Microbiol* 2007, 2(3):285-295.
6. Gentile CL, Weir TL: The gut microbiota at the intersection of diet and human health. *Science* 2018, 362(6416):776-780.
7. Bauer PV, Duca FA, Waise TMZ, Rasmussen BA, Abraham MA, Dranse HJ, Puri A, O'Brien CA, Lam TKT: Metformin Alters Upper Small Intestinal Microbiota that Impact a Glucose-SGLT1-Sensing Glucoregulatory Pathway. *Cell Metab* 2018, 27(1):101-117 e105.
8. Bauer PV, Duca FA, Waise TMZ, Dranse HJ, Rasmussen BA, Puri A, Rasti M, O'Brien CA, Lam TKT: Lactobacillus gasseri in the Upper Small Intestine Impacts an ACSL3-Dependent Fatty Acid-Sensing Pathway Regulating Whole-Body Glucose Homeostasis. *Cell Metab* 2018, 27(3):572-587 e576.
9. Martinez-Guryn K, Hubert N, Frazier K, Urlass S, Musch MW, Ojeda P, Pierre JF, Miyoshi J, Sontag TJ, Cham CM *et al*: Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe* 2018, 23(4):458-469 e455.
10. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, Hyotylainen T, Oresic M, Backhed F: Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013, 17(2):225-235.
11. Shearer MJ, Newman P: Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis. *J Lipid Res* 2014, 55(3):345-362.
12. Magnusdottir S, Ravcheev D, de Crecy-Lagard V, Thiele I: Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front Genet* 2015, 6:148.
13. Keane C, Sharma P, Yuan L, Bissett I, O'Grady G: Impact of temporary ileostomy on long-term quality of life and bowel function: a systematic review and meta-analysis. *ANZ J Surg* 2020, 90(5):687-692.
14. Booijink CC, El-Aidy S, Rajilic-Stojanovic M, Heilig HG, Troost FJ, Smidt H, Kleerebezem M, De Vos WM, Zoetendal EG: High temporal and inter-individual variation detected in the human ileal microbiota. *Environ Microbiol* 2010, 12(12):3213-3227.
15. Zoetendal EG, Raes J, van den Bogert B, Arumugam M, Booijink CC, Troost FJ, Bork P, Wels M, de Vos WM, Kleerebezem M: The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J* 2012, 6(7):1415-1426.
16. Hayashi H, Takahashi R, Nishi T, Sakamoto M, Benno Y: Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. *J Med Microbiol* 2005, 54(Pt 11):1093-1101.
17. Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, Grunberg S, Baldassano RN, Lewis JD, Li H *et al*: Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* 2014, 147(5):1055-1063.e1058.

18. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S *et al*: Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014, 11(8):506-514.
19. Oozeer R, Leplingard A, Mater DD, Mogenet A, Michelin R, Seksek I, Marteau P, Dore J, Bresson JL, Corthier G: Survival of Lactobacillus casei in the human digestive tract after consumption of fermented milk. *Appl Environ Microbiol* 2006, 72(8):5615-5617.
20. Takada T, Chinda D, Mikami T, Shimizu K, Oana K, Hayamizu S, Miyazawa K, Arai T, Katto M, Nagara Y *et al*: Dynamic analysis of human small intestinal microbiota after an ingestion of fermented milk by small-intestinal fluid perfusion using an endoscopic retrograde bowel insertion technique. *Gut Microbes* 2020, 11(6):1662-1676.
21. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB *et al*: Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell* 2018, 174(6):1388-1405 e1321.
22. Ritchie ML, Romanuk TN: A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS One* 2012, 7(4):e34938.
23. Andrade ME, Araujo RS, de Barros PA, Soares AD, Abrantes FA, Generoso Sde V, Fernandes SO, Cardoso VN: The role of immunomodulators on intestinal barrier homeostasis in experimental models. *Clin Nutr* 2015, 34(6):1080-1087.
24. DuPont AW, DuPont HL: The intestinal microbiota and chronic disorders of the gut. *Nat Rev Gastroenterol Hepatol* 2011, 8(9):523-531.
25. van Baarlen P, Troost FJ, van Hemert S, van der Meer C, de Vos WM, de Groot PJ, Hooiveld GJ, Brummer RJ, Kleerebezem M: Differential NF-kappaB pathways induction by Lactobacillus plantarum in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci U S A* 2009, 106(7):2371-2376.
26. van Baarlen P, Wells JM, Kleerebezem M: Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. *Trends Immunol* 2013, 34(5):208-215.
27. Laval L, Martin R, Natividad JN, Chain F, Miquel S, Desclee de Maredsous C, Capronnier S, Sokol H, Verdu EF, van Hylckama Vlieg JE *et al*: Lactobacillus rhamnosus CNCM I-3690 and the commensal bacterium Faecalibacterium prausnitzii A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice. *Gut Microbes* 2015, 6(1):1-9.
28. Martin R, Chamignon C, Mhedbi-Hajri N, Chain F, Derrien M, Escribano-Vazquez U, Garault P, Cotillard A, Pham HP, Chervaux C *et al*: The potential probiotic Lactobacillus rhamnosus CNCM I-3690 strain protects the intestinal barrier by stimulating both mucus production and cytoprotective response. *Sci Rep* 2019, 9(1):5398.
29. Grompone G, Martorell P, Llopis S, Gonzalez N, Genoves S, Mulet AP, Fernandez-Calero T, Tiscornia I, Bollati-Fogolin M, Chambaud I *et al*: Anti-inflammatory Lactobacillus rhamnosus CNCM I-3690 strain

protects against oxidative stress and increases lifespan in Caenorhabditis elegans. *PLoS One* 2012, 7(12):e52493.

30. Natividad JM, Lamas B, Pham HP, Michel ML, Rainteau D, Bridonneau C, da Costa G, van Hylckama Vlieg J, Sovran B, Chamignon C *et al*: Bilophila wadsworthia aggravates high fat diet induced metabolic dysfunctions in mice. *Nat Commun* 2018, 9(1):2802.
31. Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van-Hylckama Vlieg JE, Strissel K, Zhao L, Obin M *et al*: Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. *ISME J* 2015, 9(1):1-15.
32. Alvarez AS, Tap J, Chambaud I, Cools-Portier S, Quinquis L, Bourlioux P, Marteau P, Guillemard E, Schrezenmeir J, Derrien M: Safety and functional enrichment of gut microbiome in healthy subjects consuming a multi-strain fermented milk product: a randomised controlled trial. *Sci Rep* 2020, 10(1):15974.
33. Garcia-Villalba R, Gimenez-Bastida JA, Garcia-Conesa MT, Tomas-Barberan FA, Carlos Espin J, Larrosa M: Alternative method for gas chromatography-mass spectrometry analysis of short-chain fatty acids in faecal samples. *J Sep Sci* 2012, 35(15):1906-1913.
34. van der Hee B, Wells JM: Microbial Regulation of Host Physiology by Short-chain Fatty Acids. *Trends Microbiol* 2021.
35. Kleerebezem M, Binda S, Bron PA, Gross G, Hill C, van Hylckama Vlieg JE, Lebeer S, Satokari R, Ouwehand AC: Understanding mode of action can drive the translational pipeline towards more reliable health benefits for probiotics. *Curr Opin Biotechnol* 2019, 56:55-60.
36. El Aidy S, van den Bogert B, Kleerebezem M: The small intestine microbiota, nutritional modulation and relevance for health. *Curr Opin Biotechnol* 2015, 32:14-20.
37. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA: Diversity of the human intestinal microbial flora. *Science* 2005, 308(5728):1635-1638.
38. Mehta RS, Abu-Ali GS, Drew DA, Lloyd-Price J, Subramanian A, Lochhead P, Joshi AD, Ivey KL, Khalili H, Brown GT *et al*: Stability of the human faecal microbiome in a cohort of adult men. *Nat Microbiol* 2018, 3(3):347-355.
39. Maldonado-Gomez MX, Martinez I, Bottacini F, O'Callaghan A, Ventura M, van Sinderen D, Hillmann B, Vangay P, Knights D, Hutkins RW *et al*: Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. *Cell Host Microbe* 2016, 20(4):515-526.
40. Derrien M, van Hylckama Vlieg JE: Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol* 2015, 23(6):354-366.
41. Kandler O: Carbohydrate metabolism in lactic acid bacteria. *Antonie Van Leeuwenhoek* 1983, 49(3):209-224.
42. Zengler K, Zaramela LS: The social network of microorganisms - how auxotrophies shape complex communities. *Nat Rev Microbiol* 2018, 16(6):383-390.

43. Machado D, Maistrenko OM, Andrejev S, Kim Y, Bork P, Patil KR, Patil KR: Polarization of microbial communities between competitive and cooperative metabolism. *Nat Ecol Evol* 2021, 5(2):195-203.
44. D'Souza G, Shitut S, Preussger D, Yousif G, Waschina S, Kost C: Ecology and evolution of metabolic cross-feeding interactions in bacteria. *Nat Prod Rep* 2018, 35(5):455-488.
45. Slobodkin A: The Family Peptostreptococcaceae. In: *The Prokaryotes: Firmicutes and Tenericutes.* Edited by Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014: 291-302.
46. Amaretti A, Gozzoli C, Simone M, Raimondi S, Righini L, Perez-Brocal V, Garcia-Lopez R, Moya A, Rossi M: Profiling of Protein Degraders in Cultures of Human Gut Microbiota. *Front Microbiol* 2019, 10:2614.
47. Davila AM, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, Sanz Y, Tome D: Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol Res* 2013, 68(1):95-107.
48. Smith EA, Macfarlane GT: Dissimilatory amino Acid metabolism in human colonic bacteria. *Anaerobe* 1997, 3(5):327-337.
49. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J: Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* 2016, 65(1):57-62.
50. Roager HM, Hansen LB, Bahl MI, Frandsen HL, Carvalho V, Gobel RJ, Dalgaard MD, Plichta DR, Sparholt MH, Vestergaard H *et al*: Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat Microbiol* 2016, 1(9):16093.
51. Bar N, Korem T, Weissbrod O, Zeevi D, Rothschild D, Leviatan S, Kosower N, Lotan-Pompan M, Weinberger A, Le Roy CI *et al*: A reference map of potential determinants for the human serum metabolome. *Nature* 2020, 588(7836):135-140.
52. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M *et al*: Personalized Nutrition by Prediction of Glycemic Responses. *Cell* 2015, 163(5):1079-1094.

Figures

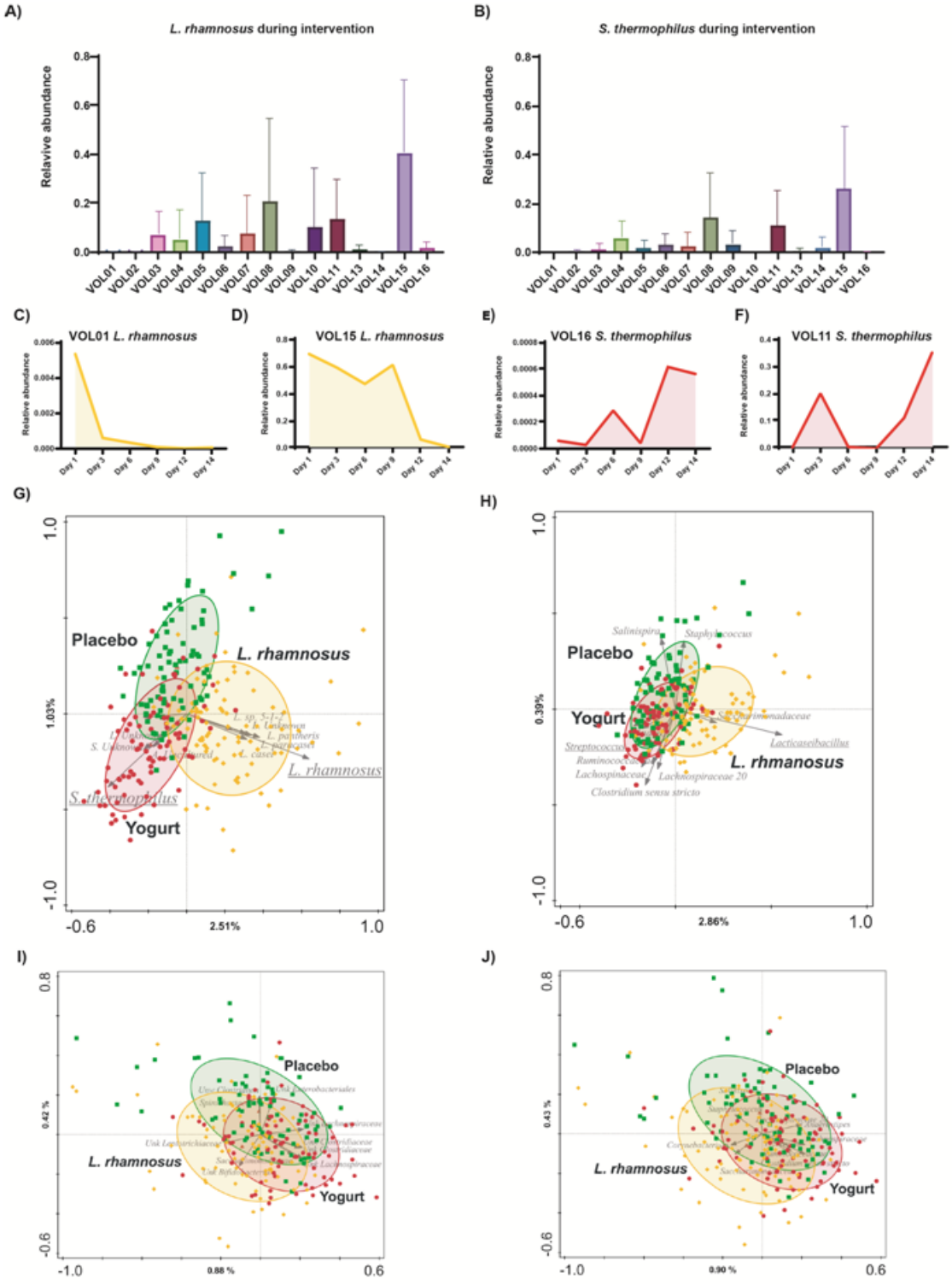

Figure 1

Microbiota impact of the consumption of fermented products. PDB could be detected in the samples obtained during the corresponding intervention period. L. rhamnosus and S. thermophilus constituted 8.5, and 5.2% of the total microbiota on average, but displayed extreme variation in intra- and inter-individual comparison (panels A till F). Subject corrected RDA analysis separated samples obtained during the different intervention periods at species (panel G, p=0.001, explained variation 2.7%) and genus (panel H,

p=0.001, explained variation 2.03%) level. Samples are coloured by intervention period (Yogurt [red], Placebo [green] and L. rhamnosus [yellow]), and the microbial species or genera with the strongest contribution to the separation are indicated, with the PDB underlined. Subject corrected RDA analysis of the microbial composition of the intervention period samples after removal of the OTUs corresponding to PDB indicated that samples could still be significantly separated on basis of the intervention period in which they were taken, albeit with much less variation explained (species level, panel I; genus level, panel J; explained variation 0.44% and 0.48%, p=0.024 and 0.036 respectively).

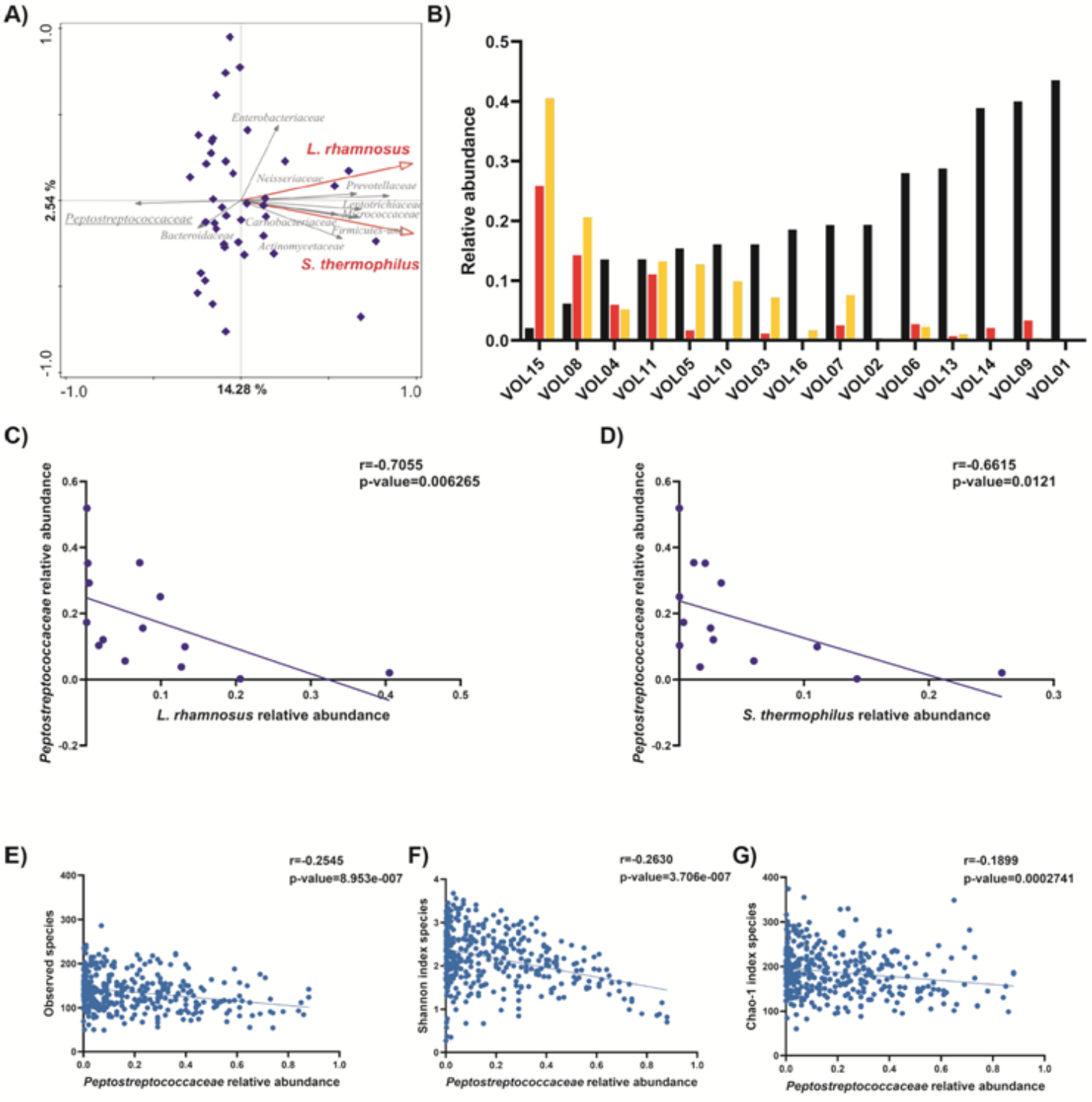

Figure 2

Association of Peptostreptococcaceae abundance with PDB and alpha diversity. Panel A: RDA analysis of the microbial composition of the run-in samples shows a negative association of participant-specific average relative abundances of L. rhamnosus and S. thermophilus during the intervention periods (indicated by the red arrows) with the Peptostreptococcaceae family relative abundance (explained variation 12.33%, p=0.002). Panel B: Per individual average relative abundance of L. rhamnosus (yellow), S. thermophilus (red), and Peptostreptococcaceae (black) in samples taken during the respective intervention periods, and the run-in period, respectively. Panel C&D: Spearman correlation of average relative abundance of Peptostreptococcaeae in the run-in samples and average relative abundance of L. rhamnosus (panel C) and S. thermophilus (panel D) in the respective intervention period samples. Panel E&F: Relative abundance of the Peptostreptococcaceae family in all samples is negatively correlated (Spearman) with microbiota alpha diversity, reflected by richness (panel E and G) and evenness (panel F).

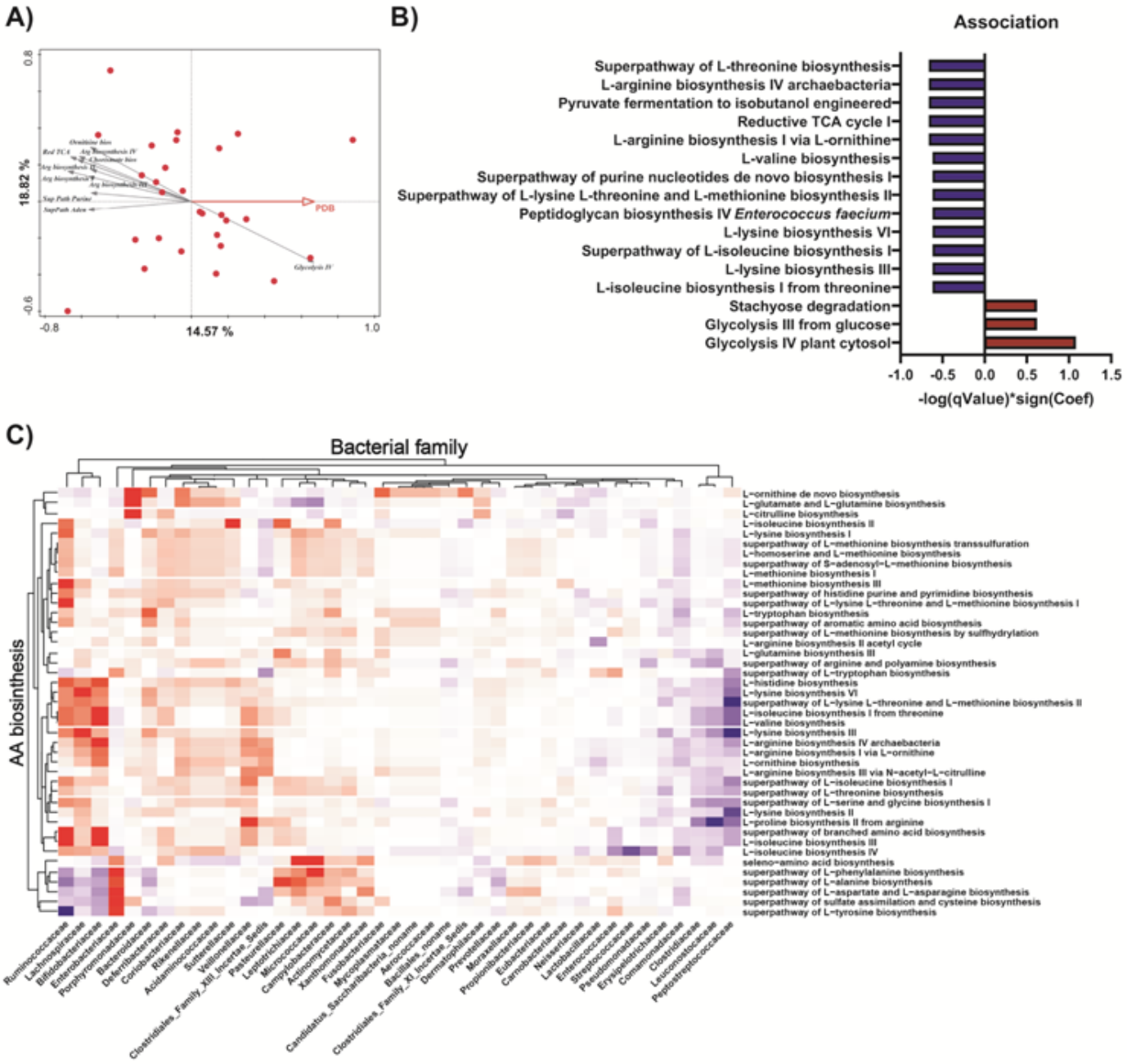

Figure 3

Small intestinal microbiota activity associates with the temporary colonization by PDB. Panel A: RDA using FMM determined during the intervention periods involving fermented products (L. rhamnosus and Yogurt) is associated with average relative abundance of PDBduring the same intervention period (red arrow), displaying top 10 strongest associated pathways (grey arrows) (explained variation: 9.83%, p=0.004.). Panel B: MaAsLin2 analysis confirmed both positive, and negative associations of PDB average abundance with microbiome activity levels of the glycolytic pathway (and expanded this observation to the Stachyose degradation pathway), and various amino acid biosynthesis pathways, respectively. Panel C: amino acid biosynthesis pathway activity associates with a variety of bacterial

families (Pearson correlation), with the notable exception of the Peptostreptococcaceae that is among the three families that negatively correlate with amino acid biosynthesis activity in the FMM.

Supplementary Files

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