UNIVERSITÀ DI PARMA

Dottorato di ricerca in Scienze degli Alimenti
Ciclo XXIX°

Microbiota modulation in human health and disease:
focus on the gut:liver:brain axis

Coordinatore:
Chiar.mo Prof. Furio Brighenti

Tutor:
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Preface

The thesis here presented resumes the three years of research activity (from September 2013 until 2016) carried out at the Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy.

The studies were supervised by Dr. Kieran M. Tuohy (formally the external tutor), head of the Nutrition and Nutrigenomics group, in the Food Quality and Nutrition Department - Research and Innovation Centre, Fondazione Edmund Mach - and Dr. Benedetta Bottari (University tutor), researcher at the Department of Food Science, University of Parma, Italy.

Summary

Intestinal microbiota dysbiosis and modification of intestinal permeability leading to bacterial translocation, have been implicated in the development of numerous liver diseases or worsening of hepatic disorders, such as cirrhosis, portal hypertension, hepatic encephalopathy (HE) and acute-on-chronic-liver failure. There is strong evidence that the pathogenesis of cirrhosis and HE is linked to a dysbiotic gut microbiota and accumulation of microbial by-products, such as ammonia, indoles, oxindoles and endotoxins, which the liver fails to detoxify. Indeed, current main line clinical treatments target microbiota dysbiosis by decreasing numbers of pathogenic bacteria and reducing blood endotoxemia and ammonia levels. Despite the large amount of existing data, there is still a need to study in more detail the composition and the metabolic output of the gut microbiota and its cross-talk with host physiological function in liver failure associated HE.

Aim of this thesis was to investigate the microbiota effects of the main current therapies used in clinical practice to treat HE. Impact of a prebiotic (lactulose), a probiotic (VLS#3) and an antibiotic (rifaximin) to modulate the gut microbiotia of cirrhotic patients both in terms of composition and metabolic output was investigated using pH controlled anaerobic batch cultures. Combining high-throughput Illumina sequencing of V3-V4 16S rRNA region, Fluorescent In Situ Hybridization coupled with flow cytometry and GC-MS, changes in faecal microbiota composition and metabolic output were measured. Significant metabolic rather than microbial changes were observed. Short chain fatty acids (acetate, propionate and acetate) production was promoted over time by lactulose and lactulose plus VSL#3 treatment and this increase was accompanied by a concomitant reduction of ammonia level and an increase in bifidobacteria. Rifaximin and its combination with lactulose was able to strongly reduce Streptococcaceae abundance, a known hallmark of cirrhotic dysbiosis, and concomitantly increase of Bifidobacteriales. Moreover I investigated how the use of VSL#3 impacted on the microbiota of paediatric patients and young adults affected by portal vein hypertension and minimal HE. VSL#3 supplementation resulted in a trend toward improved cognitive function and patients well-being. A trend towards an increased relative abundance in Actinobacteria and a

concomitant decrease in Bacteroidetes, known to be overabundant in HE dysbiosis, was observed . The results suggested also a slight increase in *Ruminococcus* and *Faecalibacterium* abundance. Indeed the data suggest an amelioration of dysbiotic condition by VSL#3 that could evolve in a decreased severity of cirrhosis progression. However, as the current pilot study was limited by sample size, these observation await confirmation in an adequately powered clinical trial.

In an effort to design more efficacious microbiota modulatory tools, I also characterized a *Lactobacillus brevis* strain isolated from an alpine traditional cheese for its potential as a next-generation probiotics thanks to its ability to produce and secrete high amounts of the neurotransmitter γ-aminobutyric acid (GABA). *Lb. brevis* FEM 1874 was able to efficiently convert glutamate to GABA by the increased expression of the GAD operon genes resulting in high GABA accumulation in the culture medium. Moreover, FEM 1874 proved resistant to acidic pH, pancreatic fluids and bile acids, good indicators for probiotic survival in the gastro-intestinal tract. FEM 1874 was also able to ferment prebiotic fibres indicating the potential of using a synbiotic formulation targeting the gut:brain axis.

Overall, the research herein showed the potential of microbiota modulatory formulations to target the dysbiosis related to gut:liver:brain axis disruption in liver disease and inducing metabolic changes capable of ameliorating related clinical symptoms.

Disseminations of results

Journal articles

Ceppa, F.; Mancini, A.; Tuohy, M., K. *Intestinal microbial fermentation patterns and their contribution to the gut:brain axis*. Article under review at International Journal of Food Sciences and Nutrition

Randazzo, C.L.; Restuccia, C.; Mancini, A.; Muccilli, S.; Gatti, M.; Caggia, C. (2016) *Ragusana Donkey Milk as a Source of Lactic Acid Bacteria and Yeast Strains of Dairy Technological Interest*. Int J Dairy Sci Process. 3(2), 38-46.DOI : dx.doi.org/10.19070/2379-1578-1600011

Lazzi C., Turroni S., Mancini A., Sgarbi E., Neviani E., Brigidi P., Gatti M. *Transcriptomic clues to understand the growth of Lactobacillus rhamnosus in cheese*. BMC Microbiol. 2014 Feb 7;14:28. doi: 10.1186/1471-2180-14-28. PMID: 24506811

Mancini, A.; Tuohy, M., K. *Gut:liver:brain axis: the microbial challenge in the hepatic encephalopathy*. Review ready for submission

Mancini, A.; Pindo, M.; D'Antiga, L.; Amodio, P.; Tuohy, M., K. *Effect of VSL#3 treatment in pediatric patients and young adults affected by Minimal Hepatic Encephalopathy from portal hypertension: a pilot intervention study*. Article ready for submission.

Mancini, A.; Campagna, F.; Amodio, P.; Pravadelli, C.; Tuohy, M., K. *Measuring cirrhotic microbiota modulation by prebiotic, antibiotic and probiotic treatment using in vitro faecal batch cultures*. Article ready for submission.

Mancini, A.; Franciosi, E.; Carafa, I.; Tuohy, M., K. *Probiotic characterization of high GABA producing strain Lactobacillus brevis FEM 1874*. Article ready for submission.

Book chapters

Bottari, B.; Mancini, A.; Ercolini, D.; Gatti, M.; Neviani, E. (2016) FISHing for food microorganisms. In Fluorescence in situ Hybridization (FISH) – Application Guide,

Edition: 2nd, Chapter: 53, Publisher: Springer, Berlin, Editors: Thomas Liher, pp.511-530 DOI: 10.1007/978-3-662-52959-1_51

Tuohy, K.; Venuti, P.; Cuva, S.; Furlanello, C.; Gasperotti, M.; Mancini, A.; Ceppa, F.; Cavalieri, D.; de Filippo, C.; Vrhovsek, U.; Mena, P.; Del Rio, D.; Fava, F. (2014) *Diet and the Gut Microbiota – How the Gut: Brain Axis Impacts on Autism*. In: Diet-microbe interactions in the gut: effects on human health and disease (editor(s) Tuohy, K.M.; Del Rio, D.). Amsterdam [et al.]: Elsevier: 225-245. ISBN: 978-0-12-407825-3 doi: 10.1016/B978-0-12-407825-3.00015-0.

Congress proceedings

4th ISM World Congress on Microbiota, abstract book in the Journal of the ISM as Journal of International Society of Microbiota, Volume 3 – Issue 1, 2016 DOI: 10.18143/JISM_v3i1

Congress presentations

October 17-19th, 2016: "4th World Congress on Targeting Microbiota" (poster presentation: Microbiota and Hepatic Encephalopathy: microbial dynamics and metabolism upon prebiotic, antibiotic and probiotic treatment). Institut Pasteur, Paris, France.

September 13-15th, 2015, "8th Probiotics, Prebiotics & New Foods - for microbiota and human health" (poster presentation: Probiotic potential of a high GABA producing strain, *Lactobacillus brevis* FEM 1874, isolated from traditional "wild" Alpine cheese). Rome, Italy,

June 5-10th, 2015: ESF-EMBO Symposium congress "Symbiomes: Systems Biology of Host-Microbiome Interactions" (poster presentation: Gut:liver:brain axis and Hepatic Encephalopathy: in vitro assessment of microbial and ammonia modulation in cirrhosis). Pultusk, Poland

February 26-28th, 2015: "EASL Monothematic Conference: Microbiota, Metabolism and NAFLD" (poster presentation: Hepatic Encephalopathy and gut microbiota: in vitro microbial and ammonia modulation by prebiotic, antibiotic and probiotic treatments). Innsbruck, Austria.

June 16-19th, 2014: attendance the congress "Gut microbiology: from sequence to function" Rowett-INRA 2014 conference (poster presentation: Probiotic potential of a BSH positive, high GABA producing strain, *Lactobacillus brevis* FEM 1874, isolated from traditional "wild" Alpine cheese). Aberdeen, Scotland (UK).

Introduction

From birth humans establish a mutualistic relationship with their gut microbiota, the composite microbial population inhabiting the gastrointestinal tract (GIT). From metagenomic studies we now know, that this complex community differs substantially in composition between individuals and that it is modulated by age, genetic background, physiological state, microbial interaction, environmental factors and diet (1–6).

Bacterial numbers within the gut microbiota reach a population of up to one hundred trillion organisms containing about 4 million distinct genes. Most of these genes encode proteins and enzymes which, even with functional redundancy, are capable of influencing the host physiology either directly or through interactions with and metabolism of human foods (7). The vast majority of these bacteria are strict anaerobes and fermentation is the main form of energy metabolism for the dominant microbiota phylotypes. Indeed, the gut microbiota may be considered an anaerobic bioreactor capable of synthesizing molecules that act directly on mammalian immune system, modify the human epigenome and regulate host metabolism (8–10). The gut microbiota uses both ingested dietary components (e.g. carbohydrates, proteins, and lipid) and host-derived components (including shed epithelial cells and mucus) to generate energy for their own cellular processes and growth and produce several metabolites which influence human health and metabolism. For instance, carbohydrate fermentation leads to the production of the short-chain fatty acids (SCFA) acetate, propionate and butyrate which contribute to normal large bowel function, immune regulation (11–16), regulation of food intake and intestinal physiology and motility (17) by regulating production of gut hormones or incretins (18), epigenetic effects through the histone deacetylase (HDAC) inhibitory activity of butyrate in particular and reducing gut wall permeability to improving tight junction control (19–21). Protein fermentation on the other hand, as well as producing some SCFA, also gives rise to phenolic metabolites and amines some of which may exert deleterious effects in the host. Gut microbiota and its metabolites have been also shown acting at the level of the enteric nervous system (ENS) (22). Moreover, it may impact also the central nervous system (CNS) and the human brain health by shaping different process.

The gut:brain axis

The gut:brain axis includes the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic nervous system (ANS), the enteric nervous system (ENS) and the gut microbiota (23,24). These components interact to form a complex bidirectional communication network whereby signals from the brain can influence the motor, sensory and secretory modalities of the gut and conversely, visceral messages from the gut can influence brain function (23,25). The data which most clearly indicates a direct influence of the gut microbiota on brain activity thus far has mainly derived from animal studies. However, the use of different laboratory animals indicate that there may be specific behavioural effects induced by specific microbiota in different mammals and the few clinical observations suggest that the influence of the gut microbiota on the gut:brain axis may also hold in humans (24). Indeed, there is an increasingly strong rationale implicating the gut microbiota in the development of the nervous system and in adverse early life influences on the gut:brain axis.

Alterations in this bidirectional gut microbiota-brain seem to be implicated as a possible mechanism in the pathophysiology of several brain disorders including autism spectrum disorders (ASDs) (26,27), Parkinson's disease (28), disorders of mood and depression (26,29), and chronic pain (30). However, the signalling mechanisms involved and how they relate to gut microbiota composition, community structure and metabolic output still remain to be determined.

Hepatic Encephalopathy

Altered metabolic, immune and hormonal homeostasis in advanced liver disease and cirrhosis may influence the onset of liver disease complications such as gut-based infections, multiorgan failure, chronic liver failure and hepatic encephalopathy (HE) (31). HE is considered a typical model of gut:liver:brain axis dysfunction, even though its pathogenesis is not well understood. Increasing evidence shows that alteration in gut microbiota and their metabolic by-products such as ammonia, indoles and/or oxindoles, a background of local and systemic inflammation, and bacterial translocation through leaky gut, may all drive the development of HE (32,33).

Even if the pathophysiological basis of HE is multifactorial and complex, there is a general consensus that ammonia plays a pivotal role (34,35). Ammonia is a common end product of amino acid fermentation by the gut microbiota and although certain groups of bacteria (e.g. the clostridia) are commonly considered responsible for amino acid fermentation in the colon, we still do not fully understand ammonia metabolism by the gut microbiota and specifically, which species/genera are involved and under what conditions ammonia is produced. Over-representation of *Streptococccaceae* and *Vellonellaceae*, with a specific overabundance of *Streptococcus salivarius*, has been observed in HE and cirrhotic patients without cognitive impairments compared to healthy controls, leading to speculation that the possible involvement of this bacterial species in ammonia production

is due to its urease activity (36). However, ammonia production, as with production of other fermentation end products, is very unlikely to be the result of the metabolism of a single species, and more likely reflects fermentation profiles and end products, cross feeding, absorption and detoxification at the community level. Recent evidence of correlations between the gut microbiota, cognition and inflammatory cytokines in HE patients derive from next generation sequencing investigations. These investigations suggest some links between relative abundance of different gut bacteria and clinical processes affecting the pathogenesis of HE, as reviewed in depth in **Chapter 1** of this thesis.

The majority of the strategies used in the treatment of HE are primarily directed at the reducing or eliminating increased neurotoxic ammonia levels (37). Consequently, most of the therapies approved and utilized to date are based on modulation of the gut microbiota. Gut microbiota modulation may have efficacy in MHE and HE by various mechanisms including a decrease in counts of pathogenic bacteria, decreased bacterial urease activity and reduced ammonia absorption by decreasing luminal pH. The most common HE treatments used in clinical practice include prebiotics, antibiotics and probiotics (38,39) as discussed in **Chapter 1**. The first line of intervention in HE is the prebiotic lactulose (4-O-β-d-galactopyranosyl-d-fructose). However, the actual mechanism by which lactulose appears to work in HE is still not fully understood. Possible mechanisms seem to be related, in part, to alterations in gut microbiota, since lowering the colonic pH is linked to production of organic acids through bacterial fermentation. Lower pH and increased organic acids can inhibit urease-producing bacteria such as *Klebsiella* spp. and *Proteus* spp., facilitating the growth of acid resistant, non-urease-producing species, such as lactobacilli and bifidobacteria thus impacting on colonic ammonia production. Similarly, by providing a readily fermentable source of carbohydrate, lactulose switches off amino acid fermentation and thus ammonia production via this route. The non-absorbable antibiotic rifaximin, has been also shown to be effective in improving cognitive function in HE and is the most commonly used antibiotic to treat HE, especially in patients who do not respond to lactulose. Again the precise mechanism of action remains unclear (40). Probiotic treatment in patients with decompensated cirrhosis and HE has been shown to reduce serum ammonia levels and improve various neurocognitive tests and mental status (41). Commonly used as a second line intervention in HE, the probiotic VSL#3 (*B. longum*, *B. infantis*, *B. breve*, *L. acidophilus*, *L. casei*, *L. delbrueckii* ssp. *bulgaricus*, *L. plantarum* and *St. salivarius* ssp. *thermophilus*) has been demonstrated to be effective in preventing HE in patients with cirrhosis, to significantly reduce the level of arterial ammonia, small intestine bacterial overgrowth (SIBO) and orocaecal transit time together with increased psychometric HE scores, compared with placebo (42).

However, due to the nature of these studies, i.e case/control studies and random controlled intervention trials, from the data available to date a clear association but not causation can be made between cognitive performance, HE and gut microbiota. Co-

occurrence has been observed between certain microbial changes and improving symptoms. The use of *in vitro* fermentation systems inoculated with human faecal samples is widely accepted to simulate environmental conditions in the human large intestine (43). Indeed, its use provides an initial model to better understand the link between microbiota relative abundance, amino acid fermentation and ammonia production. I*n vitro* systems could give insight on the fermentation profiles of the complex bacterial communities altered in HE giving insight on the mechanisms by which gut microbiota affects brain and liver function.

In **Chapter 2** of this thesis, an *in vitro* pH-controlled batch culture system has been used to study the effect of lactulose, rifaximin, VSL#3 and their combination on the gut microbiota population of cirrhotic patients. SCFA content and ammonia levels have also been correlated to the population structure analyzed by means of 16 rRNA sequencing.

The effect of the probiotic VSL#3 on gut microbiota has also been studied *in vivo* in paediatric subjects affected by portal hypertension and MHE, a study carried out at the U.S.S.D Epatologia Gastroenterologia e Trapianti pediatrici, Azienda Ospedaliera Ospedali Riuniti di Bergamo. Data are presented in **Chapter 3**.

Probiotic potential of γ-amminobutyric acid (GABA)-producing *Lactobacillus brevis*

As described in the previous section, in recent years much attention has been focused on the interaction between the intestinal microbiota, the gut, and the central nervous system (CNS) in the so called gut:brain axis (44–47). Indeed, gut microbiota modulation via probiotics represents a possible therapeutic strategy in ameliorating certain brain disorders and other systemic conditions. Bacteria commonly used as probiotics, especially bifidobacteria and lactobacilli, are able to produce a wide range of metabolites which may be involved in their probiotic potential. These metabolites include SCFA, vitamins B and K (48); bacteriocins (49), exopolysaccharides (50–52), which exert immunomodulatory function (50); conjugated linoleic acid (51–56) and also neurotransmitters like γ-amminobutyric acid (GABA) and serotonin.

GABA is a non-protein amino acid widely distributed in nature which plays an important role in the mammalian central nervous system as the major inhibitory neurotransmitter (57). Moreover GABA is involved in physiological function and is involved in induction of hypotensive, diuretic and tranquilizing effects, but also in the regulation of different neurological disorders such as Parkinson's disease, Alzheimer's disease and Huntington's chorea (58,59). Aside from CNS, GABA is present also in many organs such as the pancreas, pituitary, testes, gastrointestinal tract, ovaries, placenta, uterus and adrenal medulla (60). The potential probiotic strain *Lb. rhamnosus* (JB-1) was shown able to induce a direct effect on behavioural and physiological responses in a vagus nerve-dependent manner (61). *L. rhamnosus* (JB-1) was able to modulate the expression of receptor implicated in anxiety behaviour and responses such as GABAAα2, GABAAα1, and GABAB1b (61), leading to the speculation that the changes induced by this probiotic

strain might provide an advantage toward stressful situations. Moreover, mimicking GABA molecules or increasing environmental GABA concentration in the brain was associated with a decreased cytokine production in macrophages (62,63). The cell signalling potential of GABA in immune cells may therefore also be of importance in terms of inflammatory processes not only in the gut but systemically.

A number of different species of bifidobacteria and lactobacilli have been shown to produce GABA, in particular *Lactobacillus* subsp. isolated from fermented food (64), as shown by Siragusa et al. (65,66) with respect to *Lb. paracasei*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lc. lactis*, *Lb. plantarum*, and *Lb. brevis* strains isolated from different Italian cheese varieties. Other GABA producing LABs have been isolated from tempeh, fruit juices and fermented dairy and soy products (Higuchi et al. 1997; Nomura et al. 1998; Hou et al. 2000; Aoki et al. 2003; Inoue et al. 2003; Siragusa et al. 2007; Chang et al. 2009; Kim et al. 2009; Lim et al. 2009). Wu and co-workers (67) reported the presence or absence of glutamate decarboxylase (gad) operons in the available genome sequences of *Lb. brevis* strains in 2016. 13 out of 14 published genomes have the intact gad operon. The amino acid sequences of GADs are highly conserved at the species level, where the genes encoding GADs are mainly distributed amongst *Lactobacillus brevis*, *Lb. plantarum*, *Lb. fermentum*, *Lb. reuteri*, *Strrptococcus thermophilus*, *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* and some *Bifidobacterium* species. Most high GABA producing strains have been shown to belong to *Lb. brevis* and *Lb. plantarum*, even if species such as *Lc. lactis*, *Str. thermophilus* and *Lb. bulgaricus* isolated from milk environments also exhibit abilities to produce GABA in lower amounts (67). Also human intestinal *Lactobacillus* and *Bifidobacterium* isolates have been shown to produce GABA (68). In particular, *Lb. brevis* DPC6108 was able to significantly increase the GABA concentration of fermented faecal slurry, indicating that GABA biosynthesis could occur *in vivo* (68). *Lb. brevis* therefore, represents a promising starter for dairy fermentation to manufacture GABA-rich cultured dairy foods to be used in restoring or ameliorating conditions linked to an altered gut microbiota:(liver):brain axis.

A probiotic strain is “a live organism which when administered in adequate amounts confer a health benefit on the host” (69). An effective probiotic will maintain sufficient viable microorganisms that can survive the host's digestive process, adapt to the resident microbiota - not displacing the native bacteria already present - and produce a beneficial response in the host without pathogenic or toxic adverse effects. Indeed, a probiotic should resist the acidic environment of the stomach and the effects of bile in the duodenum (70). As already observed in Listeria monocytogenes, GAD activity in Lb. brevis may be critical for survival in acidic conditions and allows it to overcome the low pH stresses of fermented foods, gastric juice, volatile fatty acids in the GIT (75).

Indeed, the ability to convert monosodium glutamate to GABA may be considered as a novel probiotic trait, because of the beneficial health effects of GABA and its protective action to acidic pH environment.

Chapter 4 of this thesis presents the data related to the characterization of *Lb. brevis* FEM 1874 strain isolated from traditional alpine cheese for its ability to accumulate high levels of GABA in the culture medium and for some phenotypic traits important for probiotics. This preliminary characterization indicates the potential of this strain as a next-generation probiotic targeting the gut:brain axis, portal vein hypertension and systemic inflammation through GABA production.

Aim and objectives

The main hypothesis of the present thesis is if the modulation of the gut microbiota by using prebiotic, probiotic or antibiotic administration could benefit the gut:brain:axis.

To address this point:

- I reviewed the most recent literature about gut:brain:axis, with a special focus on cirrhosis and Hepatic Encephalopathy (**Chapter 1**);
- I characterized the *in vitro* microbiota modification in terms of population dynamics and composition induced by lactulose, rifaximin and VSL#3 in the cirrhotic environment; data have been associated also to microbial metabolism (**Chapter 2**);
- I characterized the *in vivo* microbiota modification in terms of population dynamics and composition induced by VSL#3 in paediatric and young adults affected by portal hypertention and minimal hepatic encephalopathy (**Chapter 3**);
- I characterized the cheese isolated *Lb. brevis* strain FEM 1874 for its potential probiotic traits and for its ability to produce high amount of GABA, which could in turn impact the gut:brain axis functioning (**Chapter 4**);

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Chapter 1

Gut:liver:brain axis: the microbial challenge in the hepatic encephalopathy

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Abstract

Hepatic encephalopathy (HE) is a debilitating neuropsychiatric condition often associated to acute liver failure or advanced liver cirrhosis. Advanced liver diseases are characterized by a leaky gut and systemic inflammation. There is strong evidence that the pathogenesis of HE is linked to a dysbiotic gut microbiota and to the microbial by-products, such as ammonia, indoles, oxindoles and endotoxins. Current main line clinical treatments target microbiota dysbiosis by decreasing the counts of pathogenic bacteria and reducing the endotoxemia. This review will focus on role of the gut microbiota and its metabolism in HE and advanced cirrhosis. It will present the different clinical trials testing the efficacy of prebiotics, probiotics and antibiotics used to treat HE and advanced cirrhosis through gut microbiota modulation. Despite the large amount of existing data, there is still a need to study in more detail the composition and the metabolic output of the gut microbiota and its cross-talk with the host as core factors in HE dysbiosis associated with liver failure.

1.1 Introduction

The human body is now considered a complex ecosystem within its own gut, harbouring thousands of different microbial species at different anatomical site and maintaining stable symbiotic or mutualistic relationships in health. From metagenomic studies in healthy subjects, we now know that substantial difference in gut microbial composition exists between individuals (1–3). In fact each individual has a unique gut microbiota which may be modulated by genetic background, physiological state, microbial interactions (e.g. phage), environmental factors and diet (4–6). There are more than 500 species in the gut of each individual in different societies and the number of species (richness) increases with age (7). The gut microbiome can be considered as an anaerobic bioreactor able to synthesize molecules that act directly on the mammalian immune system, modify the human epigenome and regulate the host metabolism (8–10). Indeed the gut microbiota uses ingested dietary components (e.g. carbohydrates, proteins, and lipid) and host-derived components (including shed epithelial cells and mucus) to generate energy for their own cellular processes and for growth and also to produce several metabolites which influence human health and disease risk. Diet has an important role in shaping the gut microbiota and also the flux of metabolites and neurochemicals they produce. Certain fibres and prebiotics, like inulin, fructo-oligosaccharides and lactulose, promote the production of Short Chain Fatty Acids (SCFA) acetate, propionate and butyrate. Indeed, certain fibre/prebiotics are thought responsible for maintaining a butyrogenic gut microbiota characterised by increased relative abundance of *Bifidobacterium* and possibly butyrate producing bacteria, like *Roseburia inulinivorans* and *Fecalibacterium prausnitzii* (11–15) by acting as growth substrates. These bacteria

appear to be important members of the beneficial gut microbiota and induce beneficial host immune effects (16–21), improve mucosal integrity intestinal permeability (16,18,21,22), intestinal motility (23) and sensitivity (17,24). Some species also produce bioactive compounds other than SCFA, such as folate, serotonin, dopamine and γ-aminobutyric acid (GABA) (25,26). Species from the genera *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Bacillus*, *Streptococcus* and *Enterococcus* have all been described to produce neurotransmitters (27–31). *Lactobacillus* and *Bifidobacterium* species have also been shown to induce hypothalamic pituitary adrenal (HPA) hormones, like adrenoicorticotropin) and cortisol production (32). Indeed, the gut microbiota and its metabolites have also been shown to be involved in modulating the activity in the enteric nervous system (ENS) (33,34). Astonishingly, recent studies in animal models show that the gut microbiota influences and shapes the brain development and function. In fact, it appears that the gut microbiota may impact on the central nervous system (CNS) and brain health in different ways: i) by stimulating the innate (e.g. gut permeability) and adaptive immune system, ii) by producing neuroactive metabolites, iii) by producing hormones and neurotransmitters identical to those of human origin, iv) by directly stimulating the afferent neurons of the ENS sending signals to the brain *via* the vagus nerve.

Alterations in the bidirectional communication between the brain and the gut microbiota have been implicated in the pathogenesis of well-known gut disorders such as irritable bowel syndrome (IBS) and related functional GIT disorders (35,36). They also seem to be implicated in the pathophysiology of several psychiatric conditions including autism spectrum disorders (ASDs) (27,37), Parkinson's disease (38), disorders of mood and anxiety (27,39), and chronic pain (40). In most of these disorders a shift from the conventional symbiotic gut microbiota, to a dysbiotic condition, seems to represent the trigger for pathogenesis evolution, or at least it occurs with the onset of disease (41). Gut microbiota dysbiosis has also been linked to liver pathologies such as non-alcoholic fatty liver disease (NAFLD) (42), non-alcoholic steatohepatitis (NASH) (43), alcoholic liver diseases (ALD), cirrhosis and hepatic encephalopathy (HE) (44). In the last decades many studies have described the alteration of gut microbiota in liver cirrhosis. Mechanistically the break-down of the intestinal barrier by bacteria (or bacterial molecules) and their translocation into the liver, systemic circulation or lymphatic system, has been suggested to give rise to systemic inflammation and altered brain functions (45).

Aim of this review is to describe how gut microbiota affects end-stage of liver disease, focusing on HE. Attention is also given to the main microbiota-targeted therapeutic approaches used to reverse the debilitating state, which characterizes HE.

1.2 Gut microbiota:liver:brain axis: a matter of microbial ecology, metabolism and inflammation

Although the gut microbiota clearly is altered in liver diseases, and has the potential to modulate physiological processes linked to liver disease, we still do not know which comes first, liver dysfunction or microbial dysbiosis. The gut liver-axis can be

defined as the set of anatomical and metabolic interactions between the gut and the liver. The liver receives more that 70% of blood from the gut through the portal vein and is continuously exposed to gut-derived bacteria, their components, including immune reactive molecules like lipopolysaccharide (LPS), and metabolites. Indeed, the liver has a fundamental physiological and crucial role in defence against gut-derived materials and xenobiotics which may be ingested with food (46,47). Moreover, the liver is rich in specific kinds of immune cells including natural killer (NK) cells, NK T cells, Kupffer cells and hepatic stellate cells, which are actively involved in maintaining a protective immune response and tolerance (e.g. resolution of inflammation), and in health, avoiding excessive reaction to exogenous antigens capable of inducing liver inflammation, autoimmune phenomena, fibrosis or carcinogenesis (48,49). In this context alteration of the gut:liver axis may evolve into dysbiosis of the conventional symbiotic microbiota which has in turn the potential to influence the aetiology of pre-cirrhotic and cirrhotic pathologies and systemic complications (42–44,50).

1.2.1 Cirrhosis and the gut microbiota

Cirrhosis is a pathological process by which the normal anatomical lobules of the liver are replaced by abnormal nodules separated by fibrous tissue (51). It represents the end result of various types of chronic liver disease. When decompensated e.g. the severe scarring of the liver has damaged and disrupted essential body functions, it drives the onset of the several complications like jaundice, variceal haemorrhage, ascites, or encephalopathy (52). When subjects reach the stage of cirrhosis, impairment of the gut-liver axis leads to gut inflammation, systemic inflammation, and worsening of liver disease complications, such as HE, gut-based infections such as spontaneous bacterial peritonitis (SBP) and eventually the development of multi-organ failure, known as acute on chronic liver failure (ACLF) (53). Clinically the severity of cirrhosis is measured by two scoring systems, the Child-Turcotte-Pugh (CTP, which includes serum albumin, bilirubin, prothrombin time, HE, and ascites severity) and the Model for End-Stage Liver Disease (MELD, logarithmic score of bilirubin, creatinine, and the international normalized ratio - INR- of the prothrombin time) (54,55).

The “cirrhosis dysbiosis ratio” (CDR) has been introduced by Bajaj and coworkers as a quantitative index to describe microbiota alterations accompanying cirrhosis progression, where a low index indicates dysbiosis (50). It has been defined as the ratio of *Ruminococcaceae*, *Lachnospiraceae* and *Clostridiales cluster XIV*, to *Enterobacteriaceae* and *Bacteroidaceae* taxa based on previous observation of a reduced relative abundance of the former and relatively increased abundance of the latter in cirrhosis and HE (45,56,57). CDR encompasses a set of various cirrhotic stages, being highest in controls (2.05) followed by compensated (0.89), decompensated (0.66), and hospitalized cirrhotic subjects (0.32). Thus, the severity of liver disease *per se* negatively affects the composition of the microbiota, where MELD scores are associated with a relative decrease in *Clostridiales XIV*, *Lachnospiraceae, Ruminococcaceae* and *Rikenellaceae*, and a relative overgrowth of

potentially pathogenic taxa such as *Staphylococcaceae*, *Enterococcaceae* and *Enterobacteriaceae*. Moreover patients with lower concentration of faecal *Clostridiales XIV*, *Lachnospiraceae* and *Ruminococcaceae* bear higher levels of endotoxin, underlining an association between microbial composition and endotoxin-mediated inflammation derived from Gram negative LPS (50). In general the severity of cirrhosis may be a stronger determinant of microbial abundance as observed by Chen and coworkers in Chinese cirrhotic subjects compared to healthy people (58). Patients showed a reduced abundance of Bacteroidetes and *Lachnospiraceae*, whereas Proteobacteria, *Fusobacterium* spp., *Enterobacteriaceae*, *Veillonellaceae* and *Streptococcaceae* were all increased compared to healthy subjects (58). However, from the data available to date a clear association but not causation can be made between cognitive performance, cirrhosis severity and gut microbiota dysbiosis.

1.2.2 Hepatic encephalopathy and the gut microbiota

Effects of altered microbiota in advanced liver disease and cirrhosis may impact on brain functions resulting in hepatic encephalopathy (HE). HE is considered a typical model of gut:liver:brain axis disease, even though its pathogenesis is not well understood. Increasing evidence shows that alteration in gut microbiota and their by-products such as ammonia, indoles, and/or oxindoles, as well as a background of local and systemic inflammation and leaky gut drive HE development (59,60).

HE is a spectrum of neurocognitive impairments and can be classified into three types, based on the nature of hepatic dysfunction: type A is associated with acute liver failure; type B occurs with portal-systemic shunting (bypass) without intrinsic liver disease; and type C develops in patients with cirrhosis (61). For more detail about definition and nomenclature in HE, please see the review from Dharel and Bajaj (54). Concerning type C HE, cirrhosis-related HE ranges from minimal (MHE), where patients are impaired on specialized cognitive tests, to overt HE (OHE), where patients experience mental status changes ranging from simple disorientation to coma. In the first case patients have difficulties in cognitive performance, psychomotor speed and visuo-motor coordination (62) resulting in reduced health-related quality of life, and increased progression to OHE. It has been shown that almost 80% of patients with chronic liver disease may have MHE with a fourfold higher risk of developing OHE (63). Indeed OHE is associated with decreased survival, risk of subsequent OHE episodes, and severely impacts on patient well-being (63,64). It can manifest as either episodic (when clinically overt symptoms develop over a short period of time) or persistent (continuous presence of symptoms) (65).

HE patients present a different composition of the sigmoid colonic mucosal microbiota (45). Lower *Roseburea* and higher *Enterococcus*, *Veillonella*, *Megasphaera* and *Burkholderia* among sigmoid colonic mucosal microbiota were observed in HE group compared to controls. The authors found that the genera like *Blautia*, *Fecalibacterium*, *Roseburia*, and *Dorea* correlated with good cognition and decreased inflammatory

markers, while species *Enterococcus* and *Streptococcus* and genera including *Burkholderiaceae*, *Veillonellaceae, Megaspheara*, *Rikenellaceae, Alistipes*, *Streptococcaceae*, *Alcaligenceae, Sutterella*, *Porphyromonadaceae*, and *Parabacteroides* were associated with poor cognitive performance in patients with and without OHE. Specifically *Alcaligenaceae* are able to produce ammonia by degradation of urea, potentially explaining their association with poor cognitive function. Moreover, Bajaj and colleagues demonstrated that *Enterobacteriaceae*, *Fusobacteriaceae*, and *Veillonellaceae* were positively, and *Ruminococcaceae* negatively, related to inflammation (56). The correlation between the microbiota, cognition and inflammatory cytokines in HE patients show the critical need to deepen study the gut mucosa since several important processes in the pathogenesis of HE occur at the mucosal interface rather than in the lumen, such as translocation and interactions between the gut microbiota and the immune system (66).

The influence of salivary microbiota on the distal gut was assessed by Bajaj and colleagues, considering microbial composition and function in cirrhotic patients with and without HE as well as the impact of cirrhosis in salivary defence and inflammation (67). Salivary microbiota analysis of cirrhotic subjects affected by HE showed an increase in *Enterobacteriaceae* with a concomitant reduction in *Erysipelothricaceae* with respect to no-HE patients and controls. *Enterobacteriaceae* was associated with functions related to endotoxin suggesting a role of oral microbiota toward the overall endotoxemia present in cirrhosis. Similar association have been noted before between oral microbiota as an inflammatory trigger of chronic low-grade systemic inflammation associated with metabolic disease and type 2 diabetes (68). Moreover in saliva a significantly higher relative abundance of *Prevotellaceae*, *Fusobacteriaceae*, and *Enterococcaceae* was observed in patients with cirrhosis, compared to controls. Correlation networks showed that cirrhotic salivary microbiota correlates well with a proinflammatory milieu, characterized by IL-1β and IL-6 production, and a consequent increase in secretory IgA (67).

In a case study, a male HE patient (MELD score 10) was subjected to to an faecal microbiota transplantation (FMT) every week for five weeks from a universal stool donor (69). Improvement in attention, serum ammonia and quality of life were observed despite missing treatments and need of hospitalization during the study. Cognitive improvements were not associated with an increase in the relative abundance of *Lachnospiraceae*, suggesting that other microbial taxa and metabolic activities might be involved. Of note was the fact that despite the initial improvement, the beneficial effect of FMT did not persist after FMT was discontinued, suggesting a transient beneficial effects of FMT with heterologous microbiota did not colonize the new host or that a repeated therapy would be required to maintain response (69). However, more subjects should be analysed to support and validate this evidence. In another study magnetic resonance spectroscopy and diffusion tensor imaging have been used to define linkage between microbial modification and neuronal astrocytic dysfunction in cirrhotic patients with and without HE (70). Patients with HE had a higher abundance pattern of *Staphylococcaceae*, *Enterococcaceae*,

Porphyromonadaceae and *Lactobacillaceae* compared to controls and no-HE (70). Brain MR spectroscopy manifestations of ammonia were positively linked with families such as *Streptococcacae*, *Enterobacteriaceae*, *Lactobacillaceae* and *Peptostreptococcaceae*, while negatively correlated with *Lachospiraceae*, *Ruminococcaeae* and *Clostridiales* XIV. The latter taxa are predominant in healthy control studies and mediate several important benefits, such as production of SCFA and 7-α de-hydroxylation of bile acids in hosts (58,71). With the progression of cirrhosis, reduction in *Lactobacillaceae* and *Peptostreptococcaceae* parallels an increase in potentially harmful taxa such as *Streptococcacae* and *Enterobacteriaceae* (72). Cognitive dysfunction correlated also with an increase of *Porphyromonadaceae* (70), a bacterial group implicated in cognitive dysfunction, progression of fatty liver disease and in systemic and hepatic inflammation in animal studies (56,73,74). Interestingly, Ahluwalia and colleagues showed an increase in *Lactobacillaceae* in HE faecal samples, potentially as expansion of selected urease-producing Firmicutes as already observed in humans and mouse cirrhosis models (72,75,76).

1.3 Gut microbiota:brain axis in liver disease: mechanisms

The higher risk of microbiota dysbiosis in cirrhotic patients, with subsequent clinical implications, is principally due to the variety of pathological interactions between the liver and the gastrointestinal tract. In particular the alteration in intestinal motility, the higher gastric pH and the reduced bile acid concentrations in the colon observed in patients affected by cirrhosis, may lead to a failure in the control of bacterial intestinal growth. Cirrhosis also impairs the homeostatic role of the liver in the systemic immune response. Damage to the reticulo-endothelial system compromises the immune surveillance function exerted by Kupffer cells and sinusoidal endothelial cells and the reduced hepatic synthesis of proteins, involved in innate immunity and pattern recognition, hinders the bactericidal ability of phagocytic cells (77,78). Monocyte spreading, chemotaxis and neutrophil activity are also significantly reduced in cirrhosis compared with controls (79,80). This in turn can lead to compromise the intestinal barrier and bacterial translocation, a higher risk of intestinal bacterial infections and increased risk of liver disease decompensation (81–87)

1.3.1 Endotoxemia

A common symptom in cirrhosis is Small Intestinal Bacterial Overgrowth (SIBO), which leads to a qualitative and quantitative alteration in the microbiota composition in the upper gut (84,88–90). Defined as $\geq 10^5$ total colony-forming units (CFUs) per milliliter of proximal jejunal aspirations, SIBO is present in 59% of cirrhotic patients and is correlated with the severity of liver disease. Indeed, SIBO, mostly induced by aerobic Gram-negative enteric organisms, like *E. coli* and *Klebsiella pneumoniae* (91–93), represents a risk factor for clinical decompensation (due to bacterial translocation and endotoxemia) of liver cirrhosis, favouring encephalopathy and SBP (88,94).

The intestinal mucosal surface has the secretory and anatomical means of preventing adhesion and translocation of microorganisms, and in health represent an efficacious barrier impeding bacteria entering the circulation. Structural changes/modifications, oxidative stress, and alteration in enterocyte function have been assessed in cirrhosis patients, as source of increases in intestinal permeability (IP) or leakiness (95–97). Leaky gut may lead to the passage of toxins, antigens, or bacteria into the body (98), and is suspected to play a pathogenic role in the development of chronic liver injury (99) as well as metabolic and immune derangement associated with many chronic debilitating diseases including obesity, type 2 diabetes and autoimmune manifestations (100,101). Bacterial translocation (BT) is the migration of viable microorganisms and microbial inflammatory products (LPS, lipoteichoic acid, bacterial DNA, peptidoglycans, and fragments) across the intestinal barrier from the intestinal lumen to mesenteric lymph nodes (MLNs) and other extra-intestinal organs or sites (102,103). Normally with a physiologically intact epithelium, endogenous bacteria translocate by an intracellular route through the epithelial lining cells and then travel via the lymph to the MLNs. When the epithelium is damaged bacteria translocate via the intercellular route between the epithelial cells directly to the blood and lymph nodes (104,105). Both the frequency and the clinical consequences of BT impact greatly on chronic disease (87).

MLNs are normally sterile (105) but in cirrhosis may be subjected to translocation and replication of the endogenous gut microbiota, specially *Enterobacteriaceae*, *Enterococcus* spp and *Proteus* spp. (106). Translocated viable bacteria may induce "spontaneous" bacterial infections while the translocation of bacterial fragments may produce a pro-inflammatory state due to the release of cytokines and nitric oxide leading to endotoxemia. The rate and degree of BT depend on the severity of liver disease and the translocation of entire and viable bacteria to MLN is a characteristic of decompensated cirrhosis. Differently, the detection of bacterial DNA in the systemic circulation and/or in MLNs seems to be independent from the severity of liver disease as observed in mice (97). Together with modification in intestinal permeability and alterations of the local host immune system, bacterial overgrowth is probably a prerequisite for the development of BT. In rats it has been shown that bacteria which translocate to MLN are the same species involved in overgrowth of the intestinal lumen, although not all the bacteria present in large quantity are found in the MLN (90,107,108). In blood of cirrhotic patients, Moratalla and co-workers specifically identified bacterial DNA attributed to the bacterial species *E. coli*, *S. aureus*, *K. pneuomoniae*, *P. vulgaris*, *P. mirabilis* and *Citrobacter freundii* and associated bacterial DNA translocation with worse neurocognitive scores in the patients analysed (109). These species, especially *E. coli*, are those which most frequently cause infections and spontaneous bacteremia in cirrhotic patients (110). Inflammatory cytokines in fact contribute to the hyperdynamic circulation, portal hypertension (84), impaired liver function and impairment of coagulation (111,112).

1.3.2 Ammonia

Blood ammonia normally ranges between 35–60 μmol/l in the presence of a healthy liver. However, during liver disease, the reduced hepatic ammonia removal capability, increases two- to five fold the ammonia blood concentration with consequent increase of its levels in the brain and associated deleterious effects (113–115). Even if the pathophysiologic basis of HE is multifactorial and complex, there is a general consensus that ammonia plays a pivotal role (113,114).

Ammonia is a by-product of nitrogen metabolism, mainly produced within the gut by the enterocytes deamination of glutamine by glutaminase in the small intestine and colon, but it is also produced upon microbial degradation of amines, amino acids, purines, and urea (116,117). Hydrolysis of urea (to carbamate and ammonia) is catalysed by the microbial enzyme urease, frequently produced by Gram negative *Enterobacteriaceae*, but also many anaerobes and Gram positive bacteria (118). Microbially produced ammonia may be absorbed across the mucosal epithelium by diffusion and transported into the hepatic portal circulation, where in a healthy liver it is removed through the urea cycle. Ammonia detoxification in the liver represents the main pathway by which ammonia homeostasis is maintained in the body, even if other organs like muscle, brain (astrocytes) and kidneys also contribute to ammonia metabolism. In the setting of liver failure however, ammonia escapes detoxification in the liver and enters the systemic circulation, inducing oxidative stress by generation of free radicals and leads to the nitrotyrosination of proteins in the brain (119–121). The neurotoxicity of ammonia is linked to its potential to modify pH, and membrane potential (113). It can also alter cellular functions like metabolism, neurotransmission, and can induce brain oedema and astrocyte swelling (120), which is a common feature of cirrhosis developing HE, found both in animal models (122,123) and in patients (124).

The role of gut microbiota in the development of experimental neuroinflammation and hyperammonemia associated with cirrhosis, has been assessed using conventional mice and germ free (GF) mice (125). Significantly higher serum levels of the proinflammatory cytokines IL-1β and TNFα and ammonia were found in conventional cirrhotic mice compared to the other groups. Cirrhotic mice showed a significantly lower relative abundance of *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiales* XIV, and *Bifidobacteriaceae* and higher *Staphylococcaceae*, *Enterobacteriaceae*, and *Lactobacillaceae* in large intestinal and caecal lining. Moreover, an increase in *Enterobacteriaceae* and *Staphylococcaceae* and *Streptococcaceae* was also observed in small intestinal border (125). Conventional cirrhotic mice also showed systemic inflammation, glial and microglial activation, and neuroinflammation associated with the microbial dysbiosis. The increase in the relative abundance of *Lactobacillaceae* in cirrhotic mice mirrored previous observations in HE patients (56). Over-representation of *Streptococccaceae* and *Vellonellaceae*, with a specific overabundance of *Streptococcus salivarius*, were observed in MHE and cirrhotic patients (even though without cognitive

impairments) compared to healthy controls, suggesting the possible involvement of this bacterial species in increasing the ammonia production due to its urease activity (126).

However, while it has been consistently shown that patients with cirrhosis have higher circulating ammonia levels, the correlation between the arterial ammonia concentration and the clinical manifestation of HE has still to be confirmed (127) As such the tight mechanistic relationship between ammonia concentration and onset of HE remains to be fully established (128).

There is a general agreement that infection is an important player in HE (127,129). Systemic inflammation participates to the propagation of cerebral consequences due to ammonia toxicity both in acute liver failure (130) and cirrhosis (131). A potential functional imbalance between a systemic inflammatory response and a compensatory anti-inflammatory response may lead to multiorgan failure and death (132,133). In this context it has been shown that ammonia itself can induce neutrophil malfunction with excessive and inappropriate release of reactive oxygen species. This in turn leads to a consequent neutrophil failure to act against bacteria such as *E. coli*. This latter evidence could somehow support the effective relationship between ammonia and inflammation in the pathogenesis of HE (134).

1.3.3 Bile Acids

Composed of individual bile acid moieties, mucous, phospholipids and biliverdin, the main physiological roles of bile is the emulsification of fats, the release of fat-soluble vitamins and regulation of cholesterol metabolism in the small intestine (135). Moreover bile acids function also as systemic signalling molecules able to significantly alter host gene-expression profiles (136,137). They act as ligands to activate or repress host receptors, expressed locally on various intestinal epithelial cells and systematically, within a diverse range of organs (including both the liver and adipose tissue), such as farnesid X receptor (FXR), pregnane X receptor and vitamin D receptor (138).

Bile acids (BAs) represent also one of the major selective pressures on the gut microbiota. BAs possess direct antimicrobial properties and can also modulate the gut microbiota through indirect activation of FXR-induced anti-microbial peptide synthesis in the small bowel (139). The gut microbiota is known to convert primary BAs chenodeoxycholic acid (CDCA) and cholic acid (CA) into secondary BAs lithocholic acid (LCA) and deoxycholic acid (DCA), respectively (140), more toxic to certain bacteria than primary BAs but also with altered signalling for mammalian BA receptors (141).

It is known that a decrease in BA production is associated with a modulation of *Lachnospiraceae*, *Ruminococcaceae*, and *Clostridiales* Cluster XIV, which normally drive the SCFA production and strengthen the integrity of the gut barrier (142–144). In cirrhosis, reduction in gastric acid barrier has been shown to result in an increase of Gram-positive "oropharyngeal microbiota" (*Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Lactobacillus* spp., *Neisseria* spp., *Veillonella* spp., *Stomatococcus* spp., *Gemella* spp., *Corynebacterium* spp., *Actinomyces* spp., *Fusobacterium* spp.) in the stomach, duodenum

and proximal jejunum. Contemporarily with the reduction in small bowel motility, potentially due to the autonomic neuropathy (145) or comorbidities (diabetes, long term pharmacological therapies), the population density of normal colonic bacteria (including *Enterobacteriaceae*, *Enterococcus* spp., *Pseudomonas* spp. and *Bacteroides* spp.) tends to increase in the small intestine (89). Additionally, due to a reduced concentration of bile acids, an overgrowth of pathogenic and pro-inflammatory members of the microbiota including *Porphyromonadaceae* and *Enterobacteriaceae* has been observed (146). Patients with advanced cirrhosis have been shown to have a 5-fold decreased concentration of faecal BAs compared to controls, accompanied by a reduction in *Blautia*, *Ruminococcaceae* and the *Clostridium* cluster XIVa group, taxa containing a high proportion of 7-α-dehydroxylating bacteria (57). As the severity of cirrhosis progresses, less secondary BAs are formed with likely knock on implications both for gut microbiota community structure and physiological function regulated by BAs system like BA biosynthesis, cholesterol metabolism, glucose homeostasis and inflammation.

1.4 Treating HE through microbiota modulation

The gut microbiota and its dysbiotic evolution in advanced liver disease is considered as the main actor, after liver failure, in HE onset. The majority of the strategies used to treat HE primarily target increased neurotoxic ammonia levels (147). Consequently, most of the therapies approved and used to date in clinical practice are based on modulation of the gut microbiota. Gut microbiota modulation may have efficacy in MHE and HE by various mechanisms including a decrease in counts of pathogenic bacteria, decreased bacterial urease activity and reduced ammonia production or absorption by decreasing luminal pH (58–61). The prebiotic non-absorbable disaccharide lactulose, non-absorbable antibiotics such as rifaximin and varying combinations of probiotics and synbiotics are the main therapies currently used in clinical practice.

1.4.1 Lactulose

Lactulose is a synthetic disaccharide, formed by the monosaccharaides lactose and galactose. It is considered a prebiotic, based on the definition: “prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits” (148). An important characteristic of prebiotics, in fact, is that they are not absorbed and must mediate their activities in the gut or systematically only after fermentation by the gut microbiota. Together with other non-absorbable disaccharides, such as inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), lactulose is able to stimulate the growth and activities of specific “good” bacteria in the gut, such as bifidobacteria (149–152). Indeed, lactulose could act on the intestinal production/absorption of ammonia through several potential mechanisms (153): i) catharsis or increase in intraluminal gas formation and osmolality as well as reduction in intraluminal pH and transit time; ii) bacterial uptake of ammonia, where lactulose promotes a bifidogenetic activity and SCFA are used as a preferred substrate by

the colonic bacteria with ammonia as the nitrogen source for protein synthesis (154,155); iii) inhibition of glutaminase activity, interfering with the intestinal uptake of glutamine and its subsequent metabolism to ammonia (156).

Lactulose effect in improving quality of life and cognitive function in patients with HE has long being studied. In 2014, the European and American Associations for the Study of the Liver (EASL/AASLD) published a joint practice guideline in which they recommended lactulose as the treatment of choice for overt hepatic encephalopathy and for secondary prevention after an index event (157). Compared to placebo or no intervention, lactulose significantly reduced the risk of no improvement in neuropsychological tests and progression to OHE, reduced blood ammonia levels and improved health-related quality of life, even if no significant difference was observed in the mortality of patients with MHE (158). Lactulose has also been shown to be effective in the treatment of MHE in patients with extra hepatic portal vein obstruction (159) and to reduce arterial ammonia, inflammatory mediators (TNFα, IL-6, IL-18) and serum endotoxin (160). A recent meta-analysis on a total of 38 randomized clinical trials involving 1828 participants provided moderate quality evidence that use of lactulose is associated with beneficial effects on hepatic encephalopathy in terms of mortality and serious adverse events when used to treat overt hepatic encephalopathy, minimal hepatic encephalopathy and to prevent hepatic encephalopathy (153). In a rat model of early HE, intragastrically administration of lactulose increased the number of new neurons in the hippocampal dentate gyrus promoting neuronal growth, showing that increased neuroplasticity may be linked to improved cognitive function (161). Moreover, lactulose also appear to exert a neuroprotective effect by elevating the number of GFAP-immunoreactive cells (161).

Despite its effect on ammonia production and its clear amelioration of HE clinical symptoms, contrasting evidences link the effect of lactulose on HE and the changes in the gut microbiota. It has been observed that lactulose treatment in patients who developed HE leads to lower CDR and an increase in relative abundance of *Enterobacteriaceae* and *Bacteroidaceae* (72). Lactulose suspension determined a reduction of *Faecalibacterium* (162). This reduction was previously observed in patients with and without recurrent HE post-withdrawal (133).

Furthermore it was demonstrated that lactulose treatment in MHE patients was able to significantly reduce the bacterial-DNA translocation rate, with consequent decrease in serum ammonia levels and an improvement in neurocognitive scores (109). Translocation of pathological bacterial antigens was present in up to one-third of MHE patients, whereas the use of lactulose significantly reduced this rate by up to 16%. This effect was also observed in a rat model of acute liver failure (163), suggesting that this disaccharide may inhibit BT (109) and reverse HE symptoms associated to gut microbiota dysbiosis by accelerating intestinal transit and improving intestinal bacterial overgrowth and permeability.

1.4.2 Rifaximin

Rifaximin is a nonsystemic structural analogue of rifamycin that inhibits the synthesis of bacterial RNA by binding to the β-subunit of bacterial DNA-dependent RNA polymerase (164,165). It effects a variety of intestinal aerobic and anaerobic bacteria (166,167). Less than 1% is absorbed after oral administration, resulting in greater concentration in the gastrointestinal tract and also presenting minimal side effects (168). At moderate and low doses it induces minimal effects on the normal gut microbiota. Higher doses, however, have been shown to initially suppress population of *Enterococcus*, *E. coli*, *Lactobacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp. and *Clostridium perfringens*, which usually return to initial values after a wash-out period in patients with ulcerative colitis (169).

In patients with cirrhosis and symptoms of HE, rifaximin has been shown to reduce serum ammonia and significantly improve neurological signs and symptoms of OHE, prevent episodes of HE and decrease the risk of hospitalization (170). It has also been shown to ameliorate acute HE (171). Rifaximin has been also used in randomised and open-label long-term studies both in the case of acute episodes and for the prevention of HE recurrence, including several studies showing beneficial effects on the neuropsychiatric and neuromotor abnormalities associated with cirrhosis (172–174). By analysing data from patients initially treated with placebo who crossed over to receive rifaximin during an open-label maintenance (OLM) study, Bajaj and colleagues (175) confirmed the efficacy of rifaximin in protecting against HE recurrence. 65% of patients who experienced an OHE episode during the placebo treatment in the randomised, controlled trial (RCT) were subsequently protected from a recurrent episode during the rifaximin therapy in the OLM study (175).

Short-term treatment with rifaximin has been shown to effectively reduce blood ammonia level and improve psychometric test, with reduction in SIBO (176). Moreover rifaximin seemed to have direct effects on intestinal barrier function and the metabolome (177,178). A correlation network study between metabolic and microbial changes upon rifaximin treatment showed correlations amongst metabolic functions associated to *Porphyromonadaceae*, *Bacteroidaceae* and *Enterobacteriaceae*, cirrhosis, MHE and cognitive dysfunction (179). In contrast, modification in faecal microbiota composition were modest with respect to the changes observed in bacterial function. Rifaximin led to an increase of *Eubacteriaceae* and beneficial species linked with less oxidative stress and aromatic amino acid and nitrogen production. A concomitant reduction in the faecal *Veillonellaceae* content was also observed (179). Abundance of *Veillonellaceae* in faeces and colonic mucosa of cirrhotic patients were confirmed in other studies and correlate with the presence of HE and MHE (56,180). *Veillonellaceae* reduction could be explained by its symbiotic relationship with taxa such as *Streptococcaceae*, which are reduced by rifaximin. Indeed the main *Streptococcaceae* end-product of metabolism is lactate, the major fermentative substrate for *Veillonellaceae*.

Very recently, Kang and co-workers (172) considered different potential effects of rifaximin in GF mice humanized with faeces from MHE patients. Specifically they measured the effect of rifaximin on intestinal ammonia and amino-acid metabolism, intestinal barrier function, dysbiosis, and systemic inflammatory milieu. Their aim was to determine if these activities were dependent upon modulation of the gut microbiota (172). An increase in the relative abundance of the families *Porphyromonadaceae* and a decrease in *Erysipelothriceae* was observed in the rifaximin-treated humanized mouse group. Moreover, rifaximin profoundly reduced the elevated serum endotoxin after humanization. Concomitantly the addition of rifaximin significantly reduced BA deconjugation and 7α de-hydroxylation in the humanized mice, resulting in conjugated BAs, secondary BAs, and the secondary/primary BA ratio being significantly lower after rifaximin therapy (172). It also improved the systemic and intestinal inflammatory cytokines e.g. IL-6 and IL-8 and increased cecal amino acids related to the urea cycle in the humanized mice. Rifaximin was also able to act on intestinal ammonia generation in the absence of the intestinal microbiota, *via* suppression of small-bowel glutaminase (172).

Overall, the studies to date available support a mode of action of rifaximin in ameliorating HE that involves modulation of bacterial metabolic function rather than reduction in overall or relative bacterial abundance. In a prospective randomized placebo study on 120 cirrhotic subjects, Sharma and co-workers investigated the synergistic effects of rifaximin and lactulose treatment on patients with OHE. The combined therapy was more effective than lactulose alone in complete resolution of HE (76% and 44% respectively). Furthermore rifaximin plus lactulose was able to reduce mortality after treatment and hospital stay compared to lactulose alone (Sharma et al., 2013). The impact of rifaximin plus lactulose treatment on the mucosal microbiota composition was also studied. The combination of the two significantly decreased the abundance of *Roseburia*, *Blautia* and *Veillonellaceae* and concomitantly increased *Propionibacterium* with respect to lactulose alone (Bajaj et al., 2012b). A previous similar study in 54 subjects with episodic HE revealed that the combination of lactulose and rifaximin was more effective in improving cognition and decreasing ammonia levels than either single treatment (181). These data reveal the potential of combined or synergistic strategies to improve treatment efficacies by acting at different physiological levels (182).

1.4.3 VSL#3

Probiotics represent an attractive therapeutic option among the potential treatment strategies in HE. As defined by the Food and Agriculture Organization and the World Health Organization, probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (183). Well tolerated even in cirrhotic patients (184), probiotics exert their efficacious effects by three suggested mechanisms: i) ammonia reduction in the portal blood (decreasing bacterial urease activity, intestinal permeability, ammonia absorption by lowering pH and improving nutritional status of the gut epithelium); ii) inflammation and oxidative stress reduction in the

hepatocytes; iii) reduced uptake of other toxins such as indoles, oxindoles, phenols and mercaptans (185–187). In general probiotics should possess specific traits such as resistance acidic pH, hydrochloric acid, and pancreatic juice; be able to tolerate stomach and duodenum conditions and gastric transport; and have the ability to stimulate the immune system thereby improving intestinal function by adhering to or colonizing the intestinal epithelium (188). The most utilized probiotics include strains of lactic acid producing bacilli (i.e. *Lactobacillus* and *Bifidobacterium*), nonpathogenic strains of *E. coli* (i.e. *E. coli* Nissle 1917), *Clostridium butyricum*, *Streptococcus salivarius*, nonpathogenic strain of yeast (i.e. *Saccharomyces boulardii*) and mixture of strains like VSL#3, which consists of a mixture of eight different probiotic strains - *Streptococcus salivarius* subp. *thermophilus*, *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, and *L. bulgaricus* (189,190). A meta-analysis on 9 intervention studies for a total of 120 subjects provided support for the efficacy of different probiotics - including among others VSL#3 (191,192) and *Lb. rhamnosus* GG (50) in the improvement of MHE symptoms and prophylaxis of OHE (193). In general RCTs comparing probiotics with no intervention or placebo in patients with HE showed that probiotics appear to reduce plasma ammonia concentrations, endotoxemia levels and improve MHE compared to patients treated with placebo or no intervention (50,72,193–196). In particular, Mittal and colleagues (197) reported reversal of MHE in 35% of patients treated with VSL#3 together with a decrease in arterial ammonia levels (197).

VSL#3 has been used with promising results in various GI diseases including Crohn's disease, IBS and ulcerative colitis (198–201). Feeding studies show a significant increase in faecal population of lactobacilli, bifidobacteria and *S. thermophilus*, without significant relative change in abundance of other bacterial group including faecal potentially pathogenic taxa like *Bacteroides* spp., coliforms, clostridia and enterococci (202). VSL#3 has been shown to have an effect in the inflammatory response, particularly inducing IL-10 in both human isolated lamina propria mononuclear cells and blood-derived dendritic cells and inhibiting the generation of proinflammatory T helper (Th)-1 cells *via* diminished levels of IL-12. This effect was suggested to derive specifically from the bifidobacteria species present in VSL#3 (203). Moreover this probiotic mixture appears to exert a beneficial effect on intestinal epithelial cells by reducing inflammation by inhibition of (NF)-κB activity and increasing heat shock protein (204) inducing expression of mucins (205), increasing transepithelial resistance upon pathogen challenge, stabilizing tight junction and reducing pathogen induced cell death (206). Available data suggest the protective effect of VSL#3 may be mediated by the DNA isolated from the bacteria rather than by their metabolic activity. In fact, systemic and oral administration of VSL#3 DNA ameliorates inflammatory responses by inhibiting colonic IFNγ secretion in mouse colons or systemic release of TNFα in response to *E. coli* DNA infection (207,208). Of consequence, VSL#3 could act on diminishing the systemic inflammation observed in chronic liver diseases. VSL#3 contains also Bile Salt Hydrolase-active bacterial species of *Lactobacillus* and *Bifidobacterium*. In mice colonization of gut microbiota with VSL#3

increased BA deconjugation and faecal excretion. This process is associated with increased hepatic BA neosynthesis and biliary output via repression of the enterohepatic FXR/Fgf15 axis (209). Indeed, VSL#3 treatment could restore the deficit in bile acid excretion seen in cirrhotic patients and limiting the overgrowth of pro-inflammatory members of the microbiota (57,146). Moreover, in a cirrhotic rat model, VSL#3 appeared to prevent endothelial dysfunction in the mesenteric artery (210) and reduce bacterial translocation, pro-inflammatory state and increase tight junction integrity (210–212), thus reducing the endotoxemia characterizing cirrhosis and HE diseases.

In patients with cirrhosis and decompensated cirrhosis, VSL#3 treatment was associated with improved hepatic and systemic haemodynamics as well as portal hypertension (213–215) thus improving MHE symptoms. An open-labeled RCT study involving 160 patients investigated the preventive effects of probiotic intervention in patients with liver cirrhosis, who had not experienced OHE (216). Patients who received VSL#3 were less likely to develop OHE compared to patients with no intervention, indicating that the probiotic could be effective in preventing OHE (216). The results also indicated that VSL#3 treatment was effective in reducing SIBO (216). The use of VSL#3 as secondary prophylaxis in HE was investigated also by Dhiman and colleagues in a RCT (192). Patients who had experienced and completely recovered from an episode of OHE where treated daily with VSL#3 for 6 months or a placebo. The VSL#3 treated group showed significant reduction in hospitalization over a 6-month period, a reduction in breakthrough episodes of encephalopathy, a reduction in inflammatory markers and an improvement in liver function (192).

The efficacy of VSL#3 in ameliorating MHE symptoms has been compared to that of lactulose in a RCT trial performed on 120 MHE patients. Improvement in neuropsychiatric tests associated with a reduction in serum ammonia level was evidenced in the probiotic treated group compared to control and lactulose treated patients (217). Despite different evidence exist on the positive effects of VSL#3 administration in ameliorating HE symptoms as reduction of inflammation and ammonia levels, its effect in restoring the gut dysbiosis seen in HE remain to be elucidated.

1.5 Conclusion

Given the emergent evidence that the gut microbiota plays a key role in human health and disease, there is currently great interest in manipulating its make up towards a potentially more beneficial composition or metabolic output (218–222). Restoring a compromised microbiota has been shown to ameliorate different disease symptoms and complications, including in severe advanced liver diseases such as cirrhosis and HE. Attempts have been made to increase beneficial bacterial groups such as *Bifidobacterium* and *Lactobacillus* that are perceived as exerting health-promoting properties as well as to be non-urease-producing bacteria (149,223,224). There is a substantial lack of information on changes and modulation of *Bifidibacterium* taxa during HE and during treatment of HE. Moreover despite a bifidogenetic activity of lactulose (152,225–231), most of the clinical

trials and studies about the use of disaccharide in HE have focused on the clinical outcomes such as cognition, metabolites and inflammation milieu (75,153,232) and few have focused on gut microbiota composition. The effect of a synbiotic preparation has been evaluated in a study involving 55 MHE patients receiving combination of probiotic (*Pediacoccus pentoseceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus paracasei* subspecies *paracasei* 19 and *Lb. plantarum* 2592) and a fermentable fibre mixture (β-glucan, inulin, pectin, and resistant starch) (233). The authors reported a decrease in arterial ammonia level, endotoxemia, and reversal of MHE in 50% of patients upon symbiotic treatment. Cirrhotic patients with MHE were also found a significant faecal overgrowth of potentially pathogenic *E. coli* and *Staphylococcus* species. Symbiotic treatment significantly increased the faecal content of non-urease-producing lactobacilli at the expense of these other bacterial species. Such modulation of the gut microbiota was associated with a significant reduction in blood ammonia levels and reversal of MHE in 50% of patients (233).

Up to now, the gut dysbiotic microbiota is considered the primary player for generating ammonia and intoxicating the host during a liver disease. Its modification in terms of microbial ecology and population structure has been until now considered as first line of intervention in HE. However, increasing evidence underlines that reducing the inflammatory burden in HE may be efficacious as well. Therefore, novel pharmacotherapeutic strategies targeting the evolution of bacterial translocation, endotoxemia and immune dysfunction should be taken into serious consideration. More studies are needed focusing the gut microbiota, using pre-, pro- or synbiotics administration, or selective gut decontamination with non-absorbable, non-toxic antibiotics, or faecal microbiota transplantation (179,233,234).

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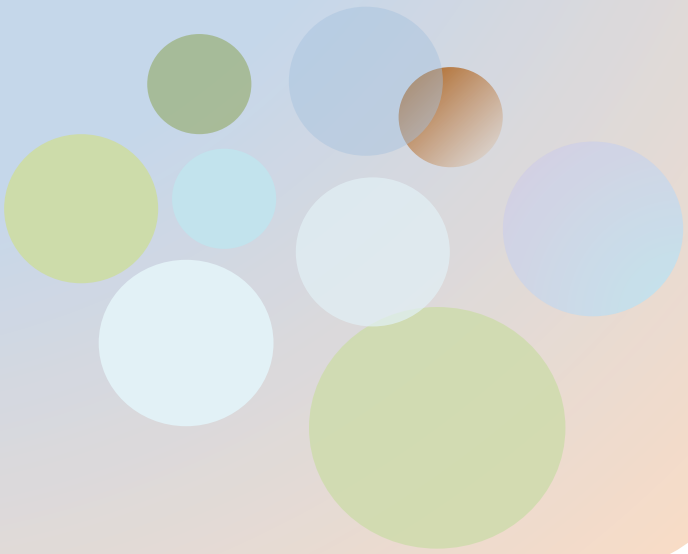
Chapter 2



Measuring cirrhotic microbiota modulation by prebiotic, antibiotic and probiotic treatment using *in vitro* faecal batch cultures

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Authors contribution:
KT conceived the idea and revised the manuscript,
AM performed sample collection, in vitro batch culture fermentation, DNA extraction, PCR amplification, FISH/FCM, ammonia concentration determination, SCFA profiling, bioinformatics data analysis and wrote the manuscript
MP performed library preparation and 16S rRNA sequencing
FC, PA, CP recruited the patients

-Paper ready for submission-

Key words
gut:liver:brain axin, Hepatic Encephalopathy, cirrhosis, lactulose, rifaximin, VSL#3, ammonia, microbiota

Abstract

Gut microorganisms may play a fundamental role in the pathogenesis and progression of liver disease and pathology. Alteration within microbiota composition and production of toxic compounds often coincide with liver pathology and may play an aetiological role. In particular, gut ammonia production from amino acid fermentation has been implicated in Hepatic Encephalopathy (HE), with a consequent strong impact on neuropsychiatric symptoms. Despite apparent clinical efficacy of prebiotics (lactulose), antibiotics (rifaximin) and probiotics (VSL#3) to ameliorate HE mental and cognitive status by reducing ammonia levels, little is known about the dynamics, interactions and responsible for metabolite production within the cirrhotic gut microbiota. We investigated how lactulose, rifaximin or VSL#3 effect gut microbial composition, ammonia and SCFA production using *in vitro* pH controlled batch cultures using faecal samples from 10 cirrhotic patients. Changes in the microbiota structure were observed at different taxonomic levels under the different test, with a particularly large increase in bifidobacteria beneficial group in lactulose fermentation. Presence of the prebiotic was also associated with acetate, propionate and butyrate production, and reduced concentration of ammonia. Further investigations are needed to associate the metabolic activity to microbial population changes and cross-talk. However, the results emphasize the importance prebiotic fermentation in shifting metabolisms of the cirrhotic microbiota towards SCFA production while reducing ammonia level.

2.1 Introduction

Recent studies have described a fundamental role for gut microbiota in the pathogenesis of liver diseases such as alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and liver cirrhosis as well as their complications such as hepatic encephalopathy (HE) (1). An alteration gut microbiota composition or dysbiosis is a characteristic of all these diseases. Small intestinal bacterial overgrowth, increased intestinal permeability, alteration in mucosal and systemic immunity, and production of toxic compounds are common disease characteristics related to the intestinal microbiota in liver disease (2). Mechanistically, higher gastric pH and reduced bile acid concentration in the colon of liver disease patients may lead to lack of control of intestinal microbiota and bacterial overgrowth. As a consequence break-down of the intestinal barrier may occur leading to bacteria (or bacterial components) translocating to the liver, systemic inflammation, and increasing the risk of liver disease decompensation and altered brain function (2–9).

Microbiota analysis of patients affected by liver cirrhosis has shown decreased relative abundance of *Bacteroidetes* in favour of increased relative abundance of

Fusobacteria and *Proteobacteria* (11–14). In particular, increased Enterobacteriaceae, Streptococcaceae and Veillonellaceae abundance in cirrhotic patients has been reported compared with healthy subjects, whereas Lachnospiraceae, Ruminococcaceae, *Blautia*, *Clostridium clusters XI and XIVab*, lactic acid bacteria, *Bifidobacterium* and *Faecalibacterium prausnitzii* seem to be present at lower levels (10,11,13,15–18). Such a modification of the gut microbiota would be consistent with a shift away from carbohydrate or fibre fermentation and increased protein or aminoacid metabolism. Additionally, due to a reduced concentration of bile acids, an overgrowth of pathogenic and pro-inflammatory members of the microbiota including *Porphyromonadaceae* and *Enterobacteriaceae* has been observed (19). It is known that a decrease in bile acid production is associated with a modulation of bacterial taxa normally involved in short chain fatty acid (SCFA) production and strengthening the integrity of the gut barrier (20–22). In these conditions, it is plausible that aberrant microbiota profiles could be deleterious to the intestinal mucosa, with consequent increase in systemic endotoxin (LPS) exposure due to the disruption of the epithelial tight junctions and imbalance of intestinal cell apoptosis (23). A compromised intestinal barrier can consequently lead to physiological effects such as hepatic and systemic inflammation and portal hypertension via immune activation (24,25).

Modified homeostasis in advanced liver disease and cirrhosis may impact on brain function, with the consequent onset of HE. Considered as a typical example of gut:liver:brain axis disruption, HE is defined by a spectrum of neurocognitive impairments ranging from minimal (MHE) to overt HE (OHE) with a strong impact on health-related quality of life (26–28). HE is characterized by a complex pathogenesis, although ammonia and other gut microbiota by-products such as indoles, and/or oxindoles and unresolved systemic inflammation to play role in disease onset (29–32). Ammonia is primarily generated in the intestine mainly from the break-down of urea by urease of colonic bacteria and during amino acid fermentation in the colon (33–35). Normally ammonia is metabolized to urea in the liver but in presence of severe liver disease, it escapes hepatic detoxification and reaches systemic circulation and the brain, altering cellular functions, metabolism, neurotransmission, and inducing brain oedema and astrocyte swelling (31,32,36–39). To decrease the production and intestinal absorption of ammonia, current HE clinical treatment is mainly based on manipulating the gut microbiota using prebiotics, antibiotics and probiotics (40,41). The prebiotic lactulose (4-O-β-d-galactopyranosyl-d-fructose) represents the first line intervention, as it lowers the colonic pH as result of production of SCFA by bacterial fermentation promoting a reduction of survival of urease-producing bacteria such as *Klebsiella* spp. and *Proteus* spp., and facilitating the growth of acid resistant, non-urease-producing species, as lactobacilli and bifidobacteria. Similarly, by providing a readily fermentable source of carbohydrate, lactulose in effect switches off amino acid fermentation and thus ammonia production. Prebiotic or lactulose treatment reduces colonic ammonia production and absorption, but also facilitates the elimination of nitrogen compounds and ammonia via microbial biomass which is then excreted (42). The

non-absorbable antibiotic rifaximin, has also been used to improve cognitive function in HE and also to prevent the development of spontaneous bacterial peritonitis and endotoxemia (and portal hypertension) in minimal HE (43). It also improves hemodynamics in cirrhotics (44). Probiotics have been utilized as adjuvant therapies for liver diseases for their putative abilities to suppress pathogenic bacteria, improve intestinal barrier function, modulate the immune system, and reduce intestinal pain perception (45–50). Moreover several probiotic studies in experimental animal models, and in a limited number of human studies in HE patients, have shown that probiotics may improve cognitive function and reduce stress and depression (51). Probiotic treatment in patients with decompensated cirrhosis and HE has been shown to reduce serum ammonia levels and endotoxemia and improve various neurocognitive tests and mental status (10,52,14,53–56). Commonly used as a second line intervention in HE, the probiotic VSL#3 (*B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. delbrueckii* ssp. *bulgaricus*, *L. plantarum* and *Streptococcus salivarious* ssp. *thermophilus*) has been demonstrated to be effective in preventing HE in patient with cirrhosis, to significantly reduce the level of arterial ammonia, small intestinal bacterial overgrowht and orocaecal transit time together with increased psychometric HE scores compared to placebo (57). Cirrhotic patients who received VSL#3 were less likely to develop overt HE indicating that the probiotic might be effective in preventing the worsening of the disease (58).

Despite the effectiveness of these therapeutic approaches in ameliorating the clinical symptoms, a clear association between the positive effect on the brain and a modulation of gut microbiota is still missing. Thus, the aim of this study was to investigate the modulation of gut microbiota, in terms of microbial populations and metabolism, upon fermentation of lactulose, rifaximin and VSL#3 using *in vitro* anaerobic pH-controlled batch cultures inoculated with faecal microbiota of cirrhotic patients or healthy subjects. Gut microbiota phylogenetic composition was measured using 16S rRNA gene community sequencing (V3-V4 region, Illumina) and selected populations enumerated using FISH. Ammonia and SCFA production over 24 hours fermentation was used to assess the impact of lactulose, rifaximin and VSL#3 and their combination on gut microbiota metabolic output.

2.2 Material and methods

2.2.1 Reagents

All media constituents were purchased from Oxoid Ltd. (Basingstoke, UK) and Sigma Aldrich (Milan, Italy), chemicals were purchased from Sigma Aldrich (Milan, Italy). VSL#3 (sachet boxes package) was kindly provided by Ferring (Milan, Italy).

2.2.2 Patients enrollment

We recruited 10 patients with clinical diagnosis of cirrhosis (median age 62, range 55-69) and 3 healthy subjects (median age 61, range 60-63) to provide faecal samples as inoculum. All subjects of this study were under their habitual diet and no antibiotics,

probiotics or prebiotics have been taken in the 3 months prior the beginning of the intervention. The study was approved by the institutional review board of the APSS Ospedale Santa Chiara (Trento, Italy), and all enrolled subjects gave written informed consent in accordance with the sampling protocol approved by the local Ethical Committee (study ID 45175518, approval N.6/2013). The characteristics of the cirrhotic population is summarized in Table 1.

Table 1. Characteristics of the cirrhotic population

	All patients (n=10)
Age [years; median (range)]	62 (55-59)
Male [n(%)]	9 (90%)
Child-Pugh	
Child-Pugh A	9 (90%)
Child-Pugh B	1 (10%)
MELD score [median (range)]	8 (7-14)
Aetiology [n(%)]	
Alcoholic cirrhosis	4 (40%)
Non Alcoholic Steatohepatitis, NASH	5 (50%)
Autoimmune cirrhosis	1 (10%)

2.2.3 Faecal batch cultures

Faecal fermentations were conducted using the basal nutrient medium prepared as follow (per litre): 2 g Peptone (Oxoid), 2 g Yeast extract (Oxoid), 2 g $NaHCO_3$ (Oxoid), 2 ml Tween 80 (AppliChem) 0.5 g Bile Salts (Oxoid), 0.1 g NaCl (Fisher Scientific), 0.04 g K_2HPO_4 (BDH), 0.04 g KH_2PO_4 (BDH), 0.01 g $MgSO_4$ $7H_20$ (BDH), 0.01 g $CaCl_2$ $6H_2O$ (Fluka), 2 ml Tween 80 (Sigma), 0.005 g Hemin (Sigma) dissolved in 1 ml of 1 M NaOH (Fisher Scientific), 10 µl Vitamin K (Sigma), 0.5 g L-Cysteine HCl (Sigma), and 1 ml of Resazurin (Sigma) (0.1 g/100ml). For each volunteer eight batch fermenters were run in parallel, filled with sterile pre-reduced PY broth and inoculated with 20 ml of 10% (w/v) faecal slurry up to a total volume of 200 ml. Slurries were prepared by homogenizing faeces in anoxic 1X PBS (pH 7.2). Anaerobic conditions were maintained by O_2-free N_2 (15 ml/min) flow overnight. Temperature was held at 37°C using a circulating water bath, and pH was controlled between 6.8 and 7.2 using an automated pH FerMac 260 controller (Gloucester, England-GL208JH, United Kingdom), which added acid and alkali as required (0.5 M HCl and 0.5 M NaOH). One gram of VSL#3 sachets content ($4.5x10^{11}$ live bacteria total), was suspended in 10 ml of anoxic PBS (pH 7.2) and cells microscopically counted with Petroff chamber. Inoculum was obtained by washing in anoxic PBS (pH 7.2) (centrifugation 4000 rpm for 7 min 3 times at 4°C each) before inoculation. The experimental conditions were as follows: vessel 1 contained only faecal inoculum (control); vessel 2 1% (w/v) lactulose; vessel 3 616 µg/ml of rifaximin; vessel 4 1% (w/v) lactulose and 616 µg/ml of rifaximin; vessel 5 VSL#3 (initial cell density 10^8cell/ml);

vessel 6 1% (w/v) lactulose and VSL#3 (initial cell density 10^8 cell/ml); vessel 7 616 μg/ml of rifaximin and VSL#3 (initial cell density 10^8cell/ml); vessel 8, 1% (w/v) lactulose, VSL#3 (initial cell density 10^8 cell/ml) and 616 μg/ml rifaximin. Each fermentation was conducted once with faecal inoculum from each of the faecal donors (n = 13). Batch cultures were run for 24 hours and samples obtained from each vessel at 0, 5, 10, 24 h, were centrifuged at 18000 rcf, where the supernatants and pellets were stored at -80 °C for metabolomics and metagenomics analysis respectively.

2.2.4 DNA extraction, PCR amplification of the V3-V5 region of bacterial 16S rDNA

Total DNA extraction from faecal samples (250 mg, wet weight) was performed using the FastDNA™ SPIN Kit for Feces (MP Biomedicals, Santa Ana, CA, USA) following manufacturer's instructions. DNA integrity and quality were checked on 1 % agarose gel TAE 1X and quantified with a NanoDrop® spectrophotometer. Samples were subjected to PCR amplification, Using the specific bacterial primer set 341F (5' CCTACGGGNGGCWGCAG 3') (59) and 806R (5' GACTACNVGGGTWTCTAATCC 3') (60) with overhang Illumina adapters targeting a ~460 bp fragment of the 16S rRNA variable region V3-V4. PCR amplification of each sample, was carried out using 25 μl reactions with 0.2 μM of each primer. In particular 12.5 μl of 2x KAPA HiFi HotStart ReadyMix, 5 μl forward primer, 5 μl reverse primer, were used in combination with 2.5 μl of template DNA (5 ng/ul). All PCR amplification was carried out, using a GeneAmp PCR System 9700 (Thermo Fisher Scientific) and the following steps: melting step – 95°C for 3 minutes (one cycle); annealing step – 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds (25 cycles); extension step – 72 °C for 5 minutes (1 cycle). The PCR products were checked on 1.5 % agarose gel and cleaned from free primers and primer dimer, using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA), following the manufacturer's instructions. Subsequently, dual indices and Illumina sequencing adapters Nextera XT Index Primer (Illumina), were attached by 7 cycles PCR (16S Metagenomic Sequencing Library Preparation, Illumina). The final libraries, after purification by the Agencourt AMPure XP system (Beckman), were analyzed on a Typestation 2200 platform (Agilent Technologies, Santa Clara, CA, USA) and quantified using the Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific). Finally, all the libraries were pooled in an equimolar way, in a final amplicon library and analysed on the Typestation 2200 platform. Barcoded libraries were sequenced on an llumina® MiSeq (PE300) platform (MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18).

2.2.5 Metataxonomic data analysis

The sequences were assigned to samples, according to sample-specific barcodes. Raw data submission to European Nucleotide Archive is pending. Reads were processed using the QIIME pipeline (61), where open reference operational taxonomic unit (OTU) picking was performed using usearch (62). Alpha (within-sample richness) and beta-

diversity (between-sample dissimilarity) estimates were computed using the phyloseq R package (63). Permutational MANOVA (PERMANOVA) was performed on the UniFrac distances and Bray-Curtis dissimilarity using the adonis function of the vegan R package with 999 permutations, and p-values were corrected using the Bonferroni correction. The non-parametric Wilcoxon rank-sum test was used for the comparison of relative abundances of microbial taxa between groups, and the resulting p-values were corrected for multiple testing controlling the false discovery rate (64) at all taxonomic levels taken into account. Starting from a table of OTUs, we obtained the final output from metagenome prediction as an annotated table of predicted gene family counts for each sample. All statistical analyses were performed using R (R: A language and environment for statistical computing, https://www.r-project.org/).

2.2.6 Fluorescence In Situ Hybridization-Flow Cytometry (FISH-FCM)

Sample fixing and hybridization were performed as previously described (65,66). Genus-specific 16S rRNA gene oligonucleotide probes labelled with the fluorescent dye Cy5 (Sigma Aldrich, Italy) were utilized for selected bacterial group, the nucleic acid stain 2-[N-(3-dimethylaminopropyl)-N-propylamino]-4-[2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methylidene]-1-phenyl-quinolinium - SYBR Green I - (Sigma Aldrich, Italy), for total cell counts. The probes used were as follows: Bif 164, specific for *Bifidobacterium* (67), Bac 303, specific for the *Bacteroides* and *Prevotella* group (68), Chis 150 for the *Clostridium histolyticum* subgroup (69), Lab 158 for *Lactobacillus* and *Enterococcus* (70), Fpra 655 for *Fecalibacterium prausnitzii* (71), Enterobac D for Enterobacteriaceae (72) and DSV 687 for Desulfovibrionales and Desulfomonales (73). Probes sequences and their respective hybridization temperatures were previously described (65). In 96 well plate, 10µl of fixed suspension was mixed into 190 µl of PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.2) and centrifuged at 1700 rcf for 15 min at 4°C. After one wash in Tris–EDTA buffer (100 mM Tris– HCl, 50 mM EDTA, pH8) when requested, pellets were suspended in Tris–EDTA buffer containing 1 mg/ml of lysozyme (Sigma Aldrich, Italy) and incubated for 10 min at room temperature. Cells were washed in PBS (pH 7.2) to remove lysozyme and were suspended in the hybridization solution (900 mM NaCl, 20 mM Tris–HCl, pH 8.0, 0.01% Sodium Dodecyl Sulfate, formamide as requested) containing 5ng/µl of specific probes, for a total volume of 55µl. The hybridization step was performed overnight at the appropriate labelled probe temperature. Following hybridization, a volume of 145µl of hybridization solution was added in each well, and cells were pelleted at 1700 rcf for 15 min, at 4°C. Nonspecific binding of the probe was removed by incubating the bacterial cell suspension at 50°C for 20 minutes in 200 µl of a washing solution (64 mM NaCl, 20 mM Tris–HCl, pH 8.0). Cells were suspended in 50 µl of PBS (pH 7.2) containing 1X SYBR Green I and incubated at room temperature for 10 minutes. Following hybridization, a volume of 150 µl of PBS (pH 7.2) was added to each well, and cells were centrifuged at

1700 rcf for 15 minutes at 4°C. Final pellet was resuspended in 100 µl of PBS (pH 7.2) and subsequently analyzed.

2.2.7 Data acquisition by flow cytometry (FCM)

The acquisition threshold was set in the side scatter channel using a Guava easyCyte 8T flow cytometer (Merck-Millipore, Italy). For each sample, a total of 10000 events were acquired. Analyses were made using the inCyte software (Merck-Millipore, Italy). Cy5- positive cells have been enumerated on SYBR green gated cells. The proportion of positive cells was corrected by eliminating background fluorescence.

2.2.8 Ammonia measurement

Ammonia concentration was spectrophotometric measured using a commercially available assay (Sigma Ammonia Assay Kit, Sigma-Aldrich, Milan, Italy), following the manufacturer instruction.

2.2.9 SCFAs analysis

Upon defrosting, 1 ml batch culture samples were centrifuged at 13.000g for 5 min. Supernatants were filtered using a 0.2-µm polycarbonate syringe filter and acidified by the addition of one volume of 6 M HCl to three volumes of sample. After 10 min incubation at room temperature, samples were centrifuged at 13.000g for 5 min. One volume of 10 mM 2-ethylbutyric acid was added to four volumes of sample as internal standard. Calibration was done using standard solutions of acetate, propionate, butyrate, isobutyrate, 2-methyl-butyrate (2-MeBut), valerate and isovalerate in acidified water (pH 2). Standard solutions containing 50, 20, 10, 5, 1 and 0.5 mM of each external standard were used. Analysis was performed using a TRACE™ Ultra Gas Chromatograph (Thermo Scientific, Waltham, MA, USA) coupled to a TSQ Quantum GC mass spectrometric detector (Thermo Scientific, Waltham, MA, USA). SCFAs were separated using a Restek Stabilwax-DA (30 m×0.25 mm; 0.25-µm film thickness) (Restek corp., Bellafonte, PA, USA). The injected sample volume was 1 µl in split mode with a ratio of 10:1. The initial oven temperature was at 90 °C and maintained for 0.5 minutes and increased 20 °C/minutes to 240 °C. The carrier gas helium was delivered at a flow rate of 1 ml min−1. The temperatures of the inlet, transfer line and electron impact (EI) ion source were set at 280, 250 and 250 °C, respectively. The electron energy was 70 eV, and the mass spectral data was collected in a full scan mode (m/z 30–200).

2.2.10 Statistical analysis

For ammonia, SCFA and FISH-FCM analysis, Kruskal Wallis test with post hoc comparison was used to compare differences between the effect of a particular treatment and the control (no treatment) at each specific time points or among the different treatments. The level of significance was set at $p<0.05$. For metagenomic data statistical analysis, please refer to paragraph 2.5.

2.3 Results

2.3.1 Microbiota community structure with faecal inoculum taken from cirrhotic patients and healthy subjects

We used *in vitro* anaerobic pH-controlled faecal batch cultures to assess the dynamics and evolution of bacterial populations of cirrhotic patients (hereinafter termed CP) in response to different treatments. The effects of the administration of the prebiotic lactulose, the antibiotic rifaximin and the probiotic VSL#3 and their combinations were determined over 24 hours, sampling at four different time points: at time of inoculation (T0) and after 5 (T5), 10 (T10) and 24 hours (T24). In parallel the same treatments were performed on healthy faecal samples (hereinafter termed HS). We characterized bacterial microbiota community structure associated with the different treatments using high-throughput sequencing of the V3-V4 region of the 16S rRNA gene.

We first quantified the bacterial richness within each sample (alpha-diversity) of the two groups, CP and HS at baseline (T0). Three different alpha-diversity estimators were used, namely the observed number of OTUs, the Chao1 index and the Shannon entropy index. Since our patients cohorts included different aetiologies leading to cirrhosis we also determined those alpha-diversity estimators considering three different CP subgroups: NASH (n=5), alcoholic cirrhosis (ALC, n=4) and autoimmune cirrhosis (AI, n=1) to exclude differences intrinsic to the pathology in determining a diverse microbiota in the CP group. No statistically difference was observed between CP and HS microbiota nor CP subgroups and HS (Figure S1, Table S1).

To identify possible differences between the bacterial components of the faecal microbiota of CP subjects vs. HS, we calculated the beta-diversity using the Unweighted and Weighted UniFrac distances and the Bray-Curtis dissimilarity (Table S2). The Principal Coordinates Analysis (PCoA) based on these measures revealed that the gut microbiota of CP subjects was different with respect to HS, however, no statistically significant differences have been found between HS and the three different CP subgroups according to aetiologies (Table S2). We next analysed which taxa, at phylum, family and order levels, were differentially represented in CP vs. HS at the baseline (T0) (Figure 1 and Table A1, Appendix A). At the phylum level the most abundant taxa were: Firmicutes (median relative abundance, CP 55%, HS 55.9%), Bacteroidetes (CP 37.07%, HS 34.83%), Actinobacteria (CP 3.35%, HS 2.87%) and Proteobacteria (CP 1.41%, HS 1.68%). At the order level, Clostridiales (CP 39.09%, HS 41.21%) Bacteroidales (CP 37.14%, HS 35.16%), Bifidobacteriales (CP 2.54%, HS 2.55%) and Lactobacillales (CP 17.94%, HS 4.49%) were the most abudant. Moving to the family level, we found: Bacteroidaceae (CP 25.74%, HS 27.43%), Lachnospiraceae (CP 19.05%, HS 18.3%), Ruminococcaceae (CP 14.31%, HS 17.94%) and Streptococcaceae (CP 11.8%, HS 2,74%). Similar levels were present in the CP subgroups according to the aetiologies (Figure 1).

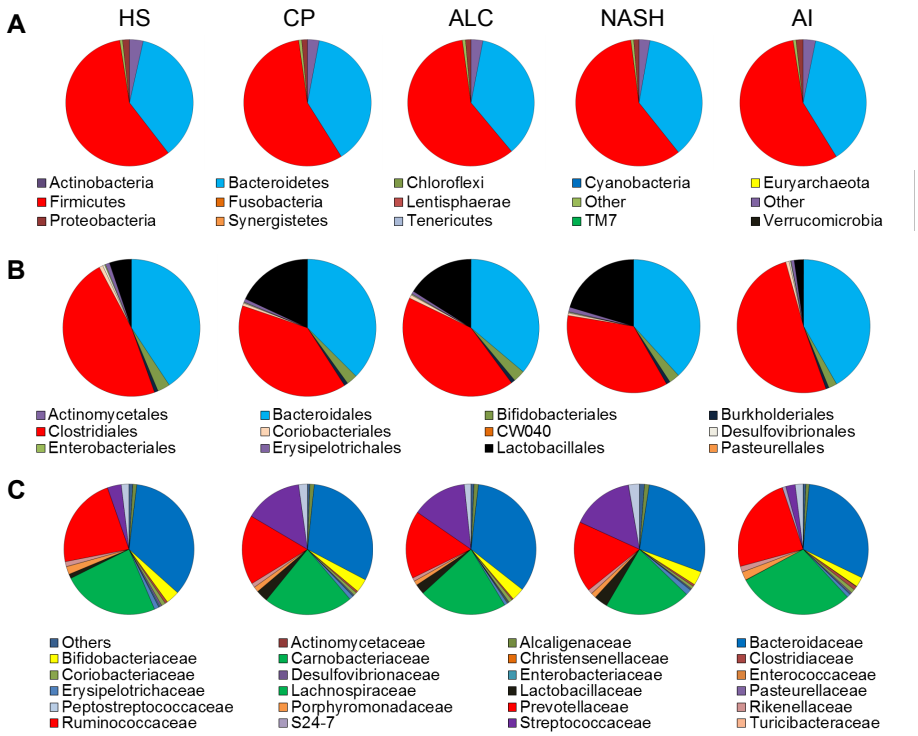

Figure 1. Representation of the most abundant bacterial phylum (A), order (B) and family (C) in Healthy subjects (HS), Cirrhotic patients (CP) and CP subgroups at the baseline. Median relative abundances expressed in percentage are presented. ALC, alcohol cirrhosis, AI, autoimmune cirrhosis, NASH, non-alcoholic steatohepatisis

Despite a trend towards an increase in Lactobacillales and Streptococcaceae in the CP, this observation was not supported statistically. However, we found that CP subjects presented a statistically significant higher abundance of *Actinomycetales* (mean relative abundance, CP 0.025%, HS 0.01%, p=0.04) and lower *Desulfovibrionales* (median relative abundance, CP 0.19%, HS 0.61%, p=0.03) and Desulfovibrionaceae (median relative abundance, CP 0.20%, HS 0.63%, p=0.009) compared to HS. We quantified the bacterial richness within each sample of the two groups CP and HS for each test fermentation at each time point. No statistically significant difference was observed comparing CP and HS microbiota during the different fermentations (Table A2, Appendix A). To identify possible differences between the bacterial components of the faecal microbiota of CP subjects vs. HS, we calculated the beta-diversity at the different time points. The Principal Coordinates Analysis (PCoA) based on these measures revealed that the gut microbiota of

CP subjects was distinct from those of the HS at T24 but only when VSL#3 was associated with Lactulose (Unweighted UniFrac $p = 0.05$, Weighted UniFrac $p \leq 0.05$, Bray-Curtis $p \leq 0.05$, PERMANOVA test) (Table A2, Appendix A). We next analyzed which taxa were differentially represented in CP vs. HS comparing them at the different time points for each fermentation condition. No statistically significant differences were observed when comparing the relative taxonomic abundances of CP and HS at the Phylum and Genus levels (Table A3-A18, Appendix A).

2.3.2 Cirrhotic microbiota change over time with respect to healthy microbiota

To understand how the cirrhotic and healthy microbiota is modulated over time, alpha and beta-diversity indices were examined longitudinally, i.e for each condition tested from T0 to T24. No significant change in microbiota composition was obtained for HS (Table S3 and S4), while evidence of a time-associated microbial dynamics, both in terms of richness and diversity, was observed in CP especially in absence of any treatment (control, ctrl) and upon treatment with lactulose + rifaximin (LR), VSL#3 + rifaximin (VR) and VSL#3 + lactulose and rifaximin (VLR) (Figure 2A, 2B and 3A, 3B, Table S5 and S6). These changes occurred at the later time points, mainly at T24, suggesting that at least ten hours are needed to observe a change in the microbiota upon probiotic, prebiotic and antibiotic modulation in this *in vitro* model.

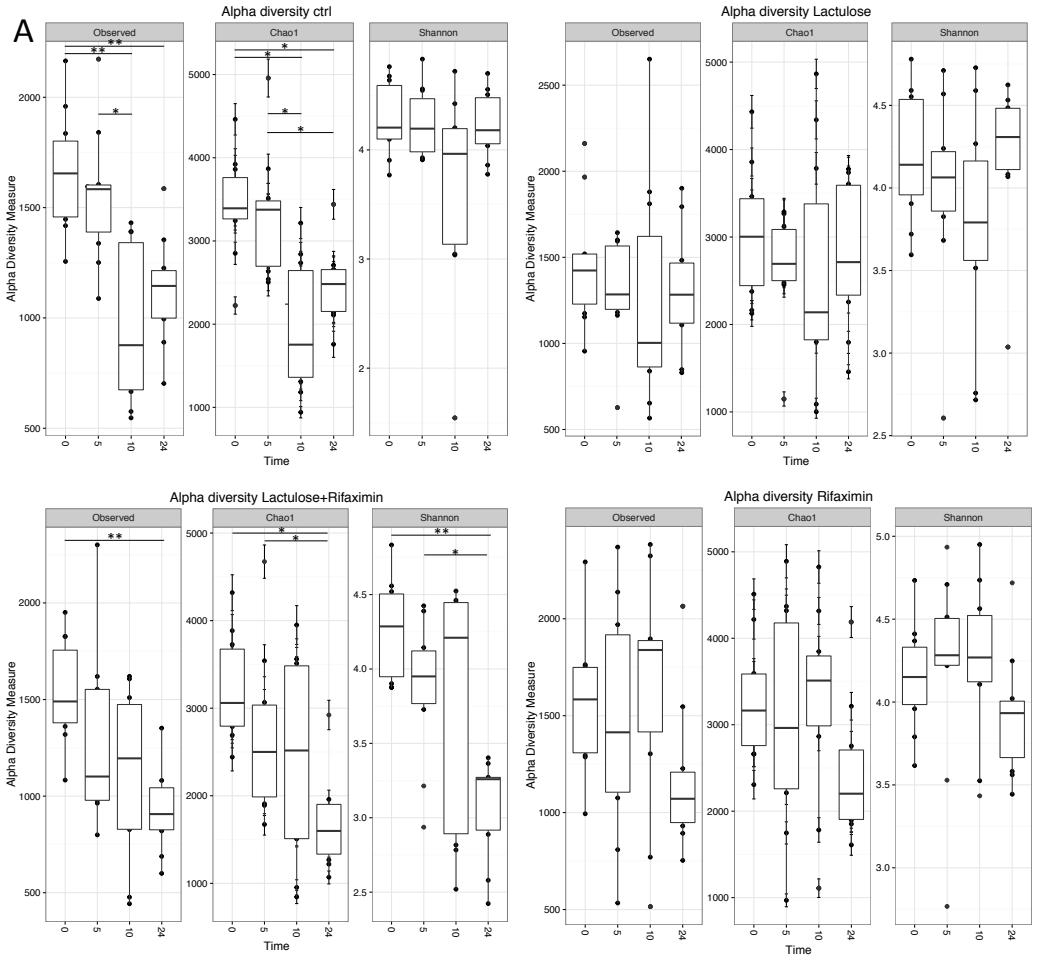

Figure 2A. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for CP subjects, overtime for each condition considered: ctrl (control), Lactulose, Lactulose+Rifaximin, Rifaximin. *p-value≤0.05, **p-value ≤0.01. The body of the box plot represents the first and third quartiles of the distribution and the median. The whiskers extend from the quartiles to the last datapoint within 1.5× IQR with outliers beyond represented as dots.

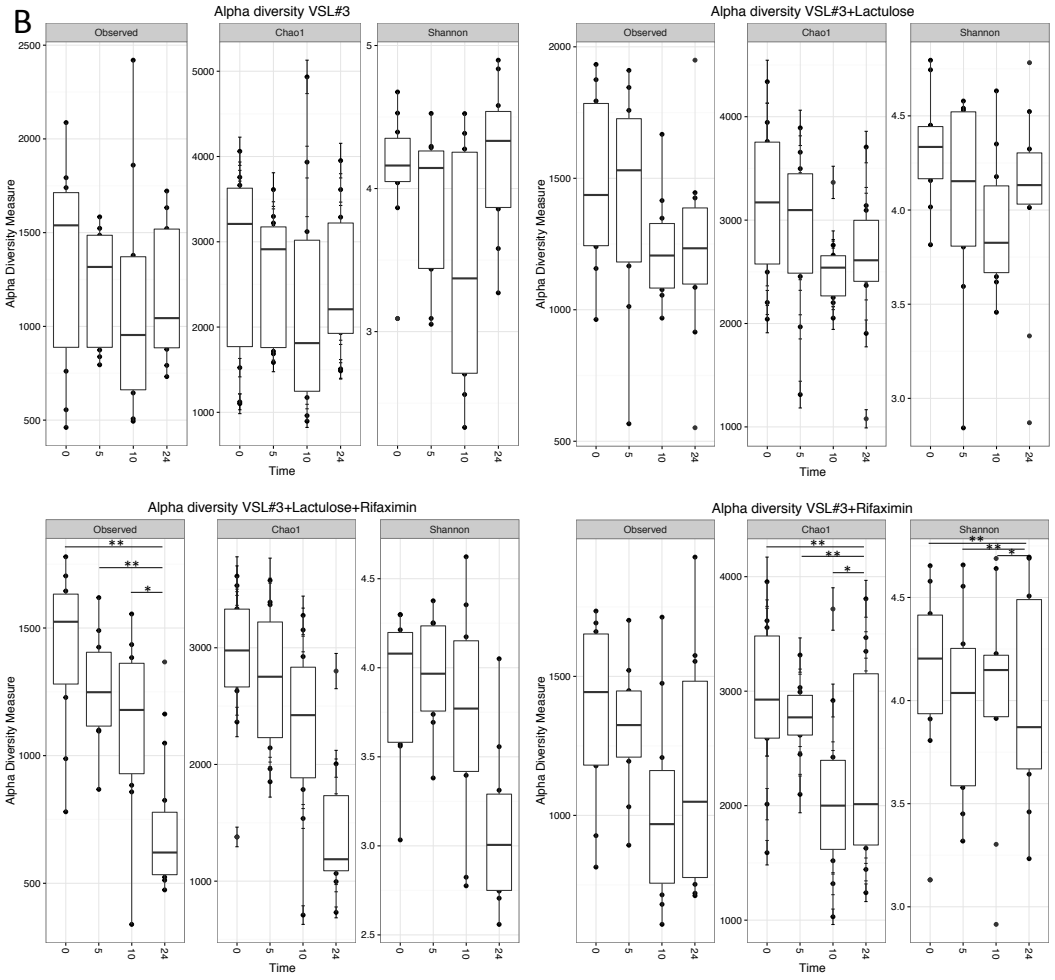

Figure 2B. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for CP subjects, overtime for each condition considered: VSL#3, VSL#3+Lactulose, VSL#3+Lactulose+Rifaximin, VSL#3+Rifaximin. *p-value≤0.05, **p-value ≤0.01. The body of the box plot represents the first and third quartiles of the distribution and the median. The whiskers extend from the quartiles to the last datapoint within 1.5× IQR with outliers beyond represented as dots.

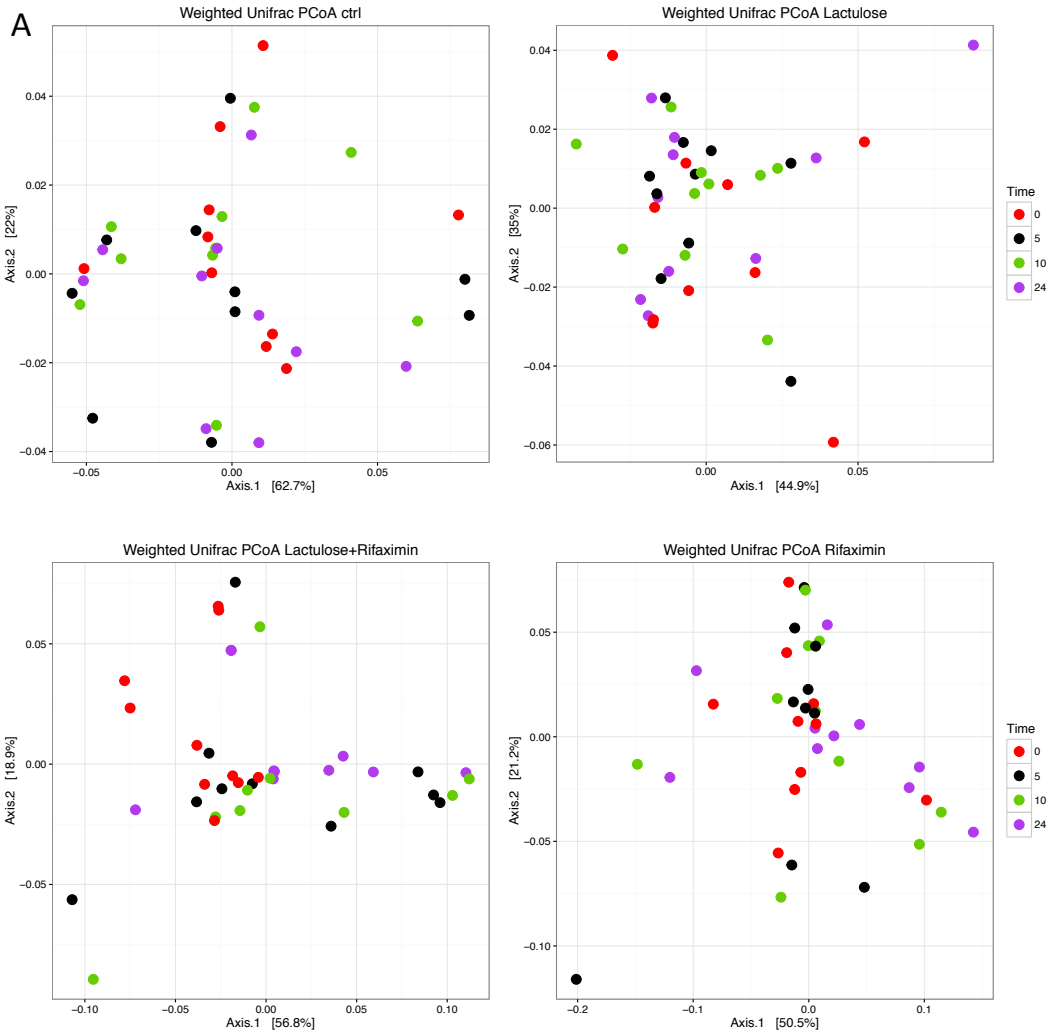

Figure 3A. PCoA plots of bacterial beta-diversity based on the Weighted UniFrac distance according to treatments in CP subjects over time: ctrl (control), Lactulose, Lactulose+Rifaximin, Rifaximin.

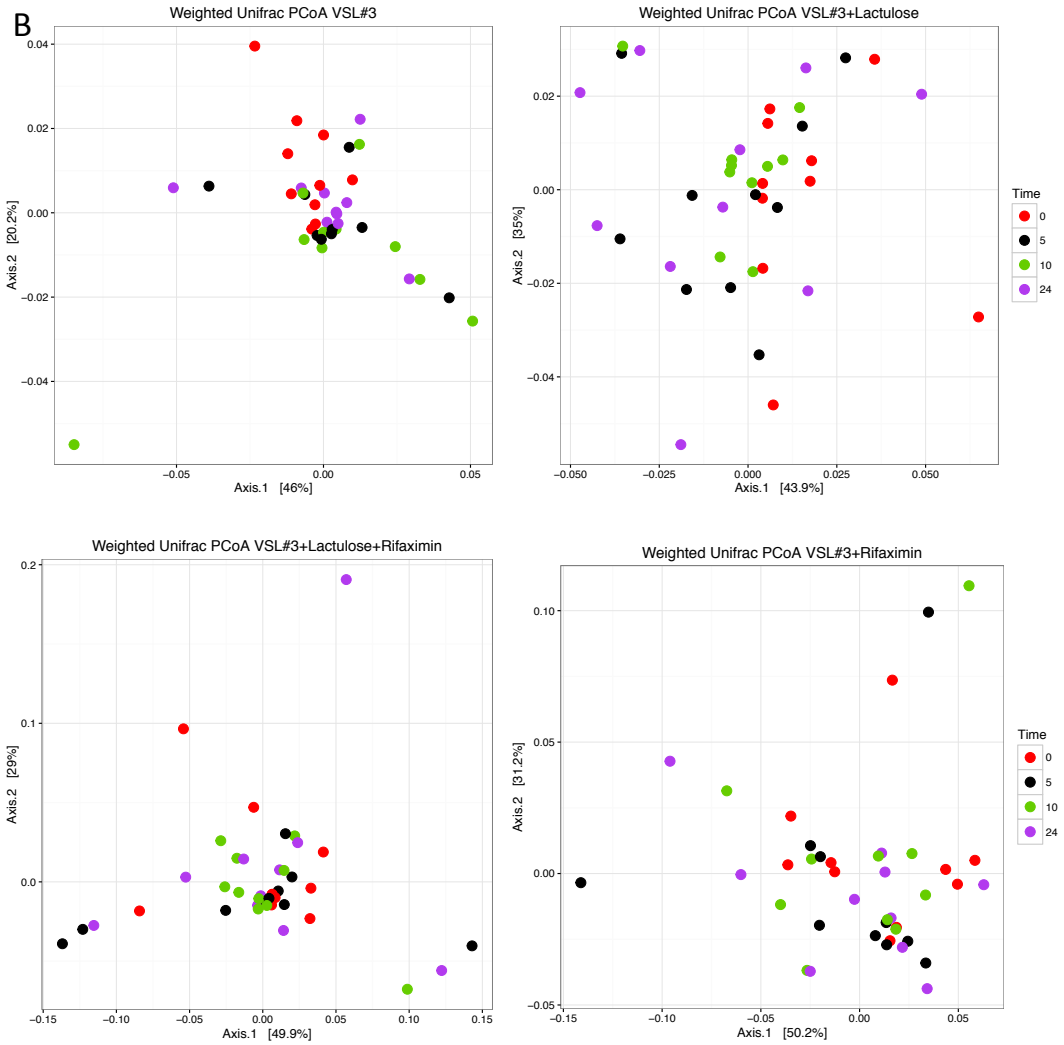

Figure 3B. PCoA plots of bacterial beta-diversity based on the Weighted UniFrac distance according to treatments in CP subjects over time: VSL#3, VSL#3+Lactulose, VSL#3+Lactulose+Rifaximin, VSL#3+Rifaximin.

We next analysed the relative abundance over time at the order, family, and genus levels (Figure 4, Table 1 and 2, Tables A3-A18 in Appendix A). Several order and family taxa are affected by the treatment over time, changing mainly after 10 and 24 hours with respect to the baseline (Table 1). Analysis of taxa abundance at genus level showed that *Bilophila* abundance increased over the 24 hours of treatment with the prebiotic, the probiotic and the antibiotic alone or combination (VL) (Table 2). In the presence of VLS#3, we found also an increased abundance of *Oscillospira*, while 24 hour-lactulose exposure decreased *Faecalibacterium*, *Odoribacter* and *Roseburia* (Table 2).

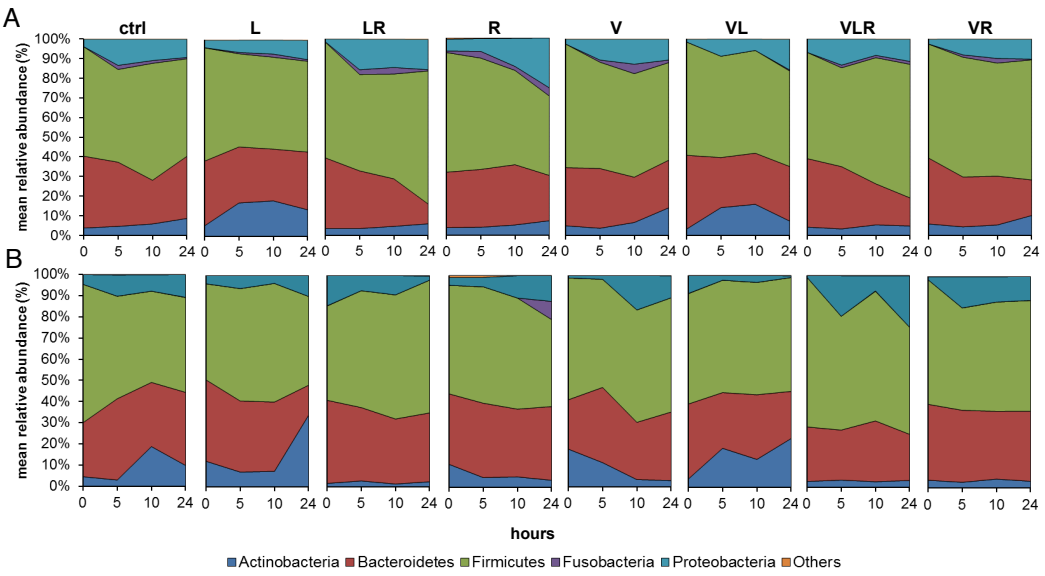

Figure 4. Representation of the most abundant bacterial phylum over time in CP (A) and HS (B) microbiota. Mean relative abundance expressed in percentage at the genus level are presented. Bacterial taxa with an abundance lower than 0.1 are included in the Others group. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

Table 1. Bacterial order and family relative abundances (expressed in %) which were significantly different (corrected $p < 0.05$; Wilcoxon rank-sum test) between the different treatments along time (T0, T5, T10 T24) within the cirrhotic environment.

Lactulose	T0 (%)	T24 (%)	p-value	T5 (%)	T24 (%)	p-value
Actinomycetales	0.03±0.02	0.01±0.02	0.020	0.06±0.10	0.01±0.02	0.04
Bacteroidales	32.92±8.52	38.56±9.49	0.01	28.4±11.33	38.56±9.49	0.04
Clostridiales	41.80±13.26	29.36±1.88	0.007	37.22±14,08	29.36±1.88	0.02
Coriobacteriales	0.84±0.65	3.51±4.26	0.04	3.55±4.04	3.51±4.26	-
Erysipelotrichales	1.58±1.11	4.23±6.17	0.007	5.06±4.97	4.23±6.17	0.028
Methanobacteriales	0.018±0.033	0.05±0.11	0.007	0.002±0.006	0.05±0.11	0.028

VSL#3 + Lactulose	T0 (%)	T24 (%)	p-value	T10 (%)	T24 (%)	p-value
Clostridiales	41.54±11.62	22.99±11.14	0.001	34.26±7.96	22.99±11.14	0.03
Coriobacteriales	0.78±0.40	1.39±1.44	0.13	2.33±2,46	1.39±1.44	0.03
Erysipelotrichales	1.50±1.61	4.17±6.15	0.14	8.47±9.13	4.17±6.15	0.03
Desulfovibrionaceae	0.23±0.18	1.71±0.78	0.008	0.53±0.59	1.71±0.78	-
Enterobacteriaceae	0.30±0.59	11.94±11.6	0.049	4.71±6.30	11.94±11.6	-

Rifaximin	T0 (%)	T24 (%)	p-value	T5 (%)	T24 (%)	p-value	T10 (%)	T24 (%)	p-value
Bacteroidales	32.13±10.7	22.97±11.06	0.0002	24.42±13.3	22.97±11.06	0.016	30.53±9.47	22.97±11.06	0.015
Clostridiales	36.56±11.2	20.14±11.03	0.0006	36.30±15.8	20.14±11.03	0.016	37,64±12.61	20.14±11.03	0.015
Lactobacillales	13.57±13.13	6.69±8.05	0.002	15.10±16.83	6.69±8.05	0.018	6.64±9.4	6.69±8.05	-
Desulfovribionaceae	0.25±0.25	5.45±3.10	-	0.96±0.82	5.45±3.10	0.049	2.84±1,62	5.45±3.10	0.042

Lactulose + Rifaximin	T0 (%)	T24 (%)	p-value	T5 (%)	T24 (%)	p-value	T10 (%)	T24 (%)	p-value
Bacteroidales	35.96±9.49	10.06±12.9	0.00005	29.34±8.65	10.06±12.9	0.001	24.19±14.9	10.06±12.9	0.009
Bifidobacteriales	2.57±2.2	5.64±12.37	0.004	2.89±3.06	5.64±12.37	0.018	3,41±6.37	5.64±12.37	0.04
Clostridiales	39.22±12.	18.96±9.7	0.000	26.77±10.	18.96±9.7	0.005	31.89±19.	18.96±9.7	0.01

Table 2. Bacterial genera relative abundances (%) which were significantly different (corrected p < 0.05; Wilcoxon rank-sum test) between the different treatments along time (T0, T5, T10, T24) within the cirrhotic environment.

Lactulose	T0 %	T24 %	p-value
Bilophila	0.11 ± 0.12	1.94 ± 1.38	0.03
Blautia	5.34 ± 2.61	5.85 ± 4.89	0.01
Faecalibacterium	4.46 ± 2.80	1.49 ± 1.51	0.01
Odoribacter	0.25 ± 0.22	0.17 ± 0.10	0.01
Parabacteroides	1.80 ± 1.69	2.90 ± 2.35	0.04
Roseburia	1.97 ± 2.46	0.09 ± 0.12	0.01
VSL#3 + Lactulose	**T0 %**	**T24 %**	**p-value**
Bilophila	0.22 ± 0.12	2.33 ± 1.27	0.02
VSL#3 + Lactulose	**T5 %**	**T24 %**	**p-value**
Bilophila	0.21 ± 0.21	2.33 ± 1.27	0.01
Rifaximin	**T0 %**	**T24%**	**p-value**
Bilophila	0.15 ± 0.10	8.35 ± 5.11	0.04
VSL#3	**T0 %**	**T24%**	**p-value**
Bilophila	0.12 ± 0.09	2.43 ± 1.49	0.04
Oscillospira	0.54 ± 0.44	2.55 ± 2.44	0.04

2.3.3 Cirrhotic microbiota respond differently to the different treatments

We next estimated for both CP and HS populations whether the different treatments affected the microbiota composition in a specific manner. We analysed the data in a cross-sectional manner, by comparing the different treatments ability to modulate the microbiota at each time point of the batch culture experiments. No differences among the treatments were observed in HS in terms of bacterial richness and diversity (Tables S25 and S26). In CP microbiota alpha-diversity estimators showed some differences between the treatments after 24 hours (T24) (Figure 5, for the complete list of p-value, refer to Table S4). Also the beta-diversity analysis conducted at each time point evidenced differences in the CP microbiota composition at T10 (Bray-Curtis p = 0.027) and T24 (Weighted UniFrac p = 0.002, Bray-Curtis p = 0.001) (Table S5). This indicated that the different treatments impacted differently the microbial diversity.

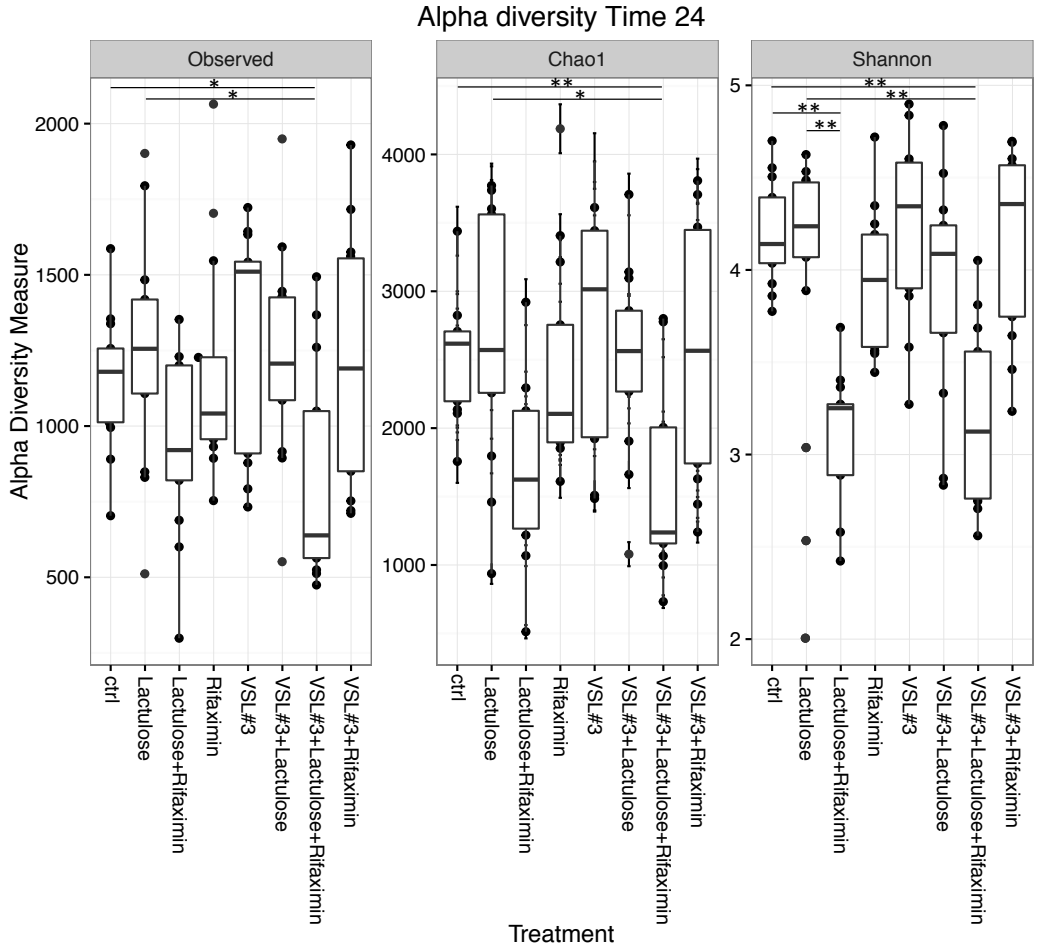

Figure 5. Measure of bacterial diversity at T24 within each test fermentation. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for CP subjects, between the conditions considered. *p-value≤0.05, **p-value ≤0.01. The body of the box plot represents the first and third quartiles of the distribution and the median. The whiskers extend from the quartiles to the last datapoint within 1.5× IQR with outliers beyond represented as dots.

Although the Bray-Curtis index found a different microbial composition at T10, no phylum, order, family or genus showed significant changes between one condition and another, suggesting that at T10 the treatments induced different alterations in the microbiota from different patients that are probably not consistent among subjects. Nevertheless at T24, rifaximin (R) and its combination with lactulose (LR) and both lactulose and VSL#3 (VLR) were able to reduce the relative abundance of some bacterial taxa, which were more abundant in the control, i.e absence of any treatment. In particular, we found differently represented: between ctrl and R, the order Coriobacteriales and Erysipelotrichales; the family Bacteroidaceae, Coriobacteriaceae, Erysipelotrichaceae, Lachnospiraceae, Reikenellaceae and Veillonellaceae, as well as the genera *Collinisella*

and *Coprococcus*; between ctrl and LR, the order Coriobacteriales and Erysipelotrichales; the family Bacteroidaceae, Coriobacteriaceae, Erysipelotrichaceae, Lachnospiraceae, and Veillonellaceae, as well as the genera *Blautia Butyricimonas*, *Collinisella Coprococcus,* and *Odoribacter*; between ctrl and VLR, the order Coriobacteriales and Erysipelotrichales; the family Coriobacteriaceae, Lachnospiraceae, and Veillonellaceae, as well as the genera *Blautia, Collinisella, Coprococcus* and *Odoribacter* (Table 3, Tables A3-A10 in Appendix A).

Table 3. Bacterial taxa which relative abundances (expressed as percentage) were significantly different ($p < 0.05$; Wilcoxon rank-sum test) at T24 in the cirrhotic environment upon the different treatments. ctrl, no treatment; R, rifaximin; LR, lactulose + rifaximin; VLR, VSL#3 + lactulose + rifaximin.

order	ctrl (%)	LR (%)	p-value
Clostridiales	31.32±10.82	31.80±6.46	0.001
Coriobacteriales	1.09±0.79	0.47±0.22	0.0003
Erysipelotrichales	8,00±11.88	31.01±0.70	0.0002

	ctrl (%)	**VLR** (%)	**p-value**
Clostridiales	31.32±10.82	21.80±3.71	0.026
Coriobacteriales	1.09±0.79	0.26±0.09	0.007
Erysipelotrichales	8.00±11.88	18.24±15.88	0.010

	ctrl (%)	**R** (%)	**p-value**
Clostridiales	31.32±10.82	32.17±13.17	0.013
Coriobacteriales	1.09±0.79	0.78±0.44	0.0008
Erysipelotrichales	8.00±11.88	30.55±3.89	0.013

family	**ctrl** (%)	**LR** (%)	**p-value**
Bacteroidaceae	24.09 ± 9.84	8.83 ± 11.28	0.040
Coriobacteriaceae	1.94 ± 1.67	0.28 ± 0.19	0.0003
Erysipelotrichaceae	4.48 ± 7.80	40.19 ± 4.81	0.0001
Lachnospiraceae	18.72 ± 4.71	6.50 ± 2.66	0.00004
Porphyromonadaceae	1.87 ± 1.29	0.98 ± 2.93	0.031
Rikenellaceae	1.68 ± 1.32	0.11 ± 0.13	0.043
S24-7	0.67 ± 1.49	0.001 ± 0.003	0.0027
Veillonellaceae	4.91 ± 3.93	0.58 ± 0.48	0.0003

	ctrl (%)	**VLR** (%)	**p-value**
Coriobacteriaceae	1.94 ± 1.67	0.70 ± 0.62	0.004

Lachnospiraceae	18.72 ± 4.71	10.26 ± 4.86	0.002
Veillonellaceae	4.91 ± 3.93	1.68 ± 1.12	0.004

	ctrl (%)	**R (%)**	**p-value**
Coriobacteriaceae	1.94 ± 1.67	0.19 ± 0.19	0.0007
Erysipelotrichaceae	4.48 ± 7.80	34.95 ± 19.26	0.013
Lachnospiraceae	18.72 ± 4.71	6.02 ± 3.49	0.0004
Veillonellaceae	4.91 ± 3.93	0.37 ± 0.35	0.004

genera	**ctrl** (%)	**LR** (%)	**p-value**
Blautia	4.69 ± 2.32	1.70 ± 1.26	0.004
Butyricimonas	0.16 ± 0.21	0.018 ± 0.034	0.04
Collinsella	2.04 ± 2.26	0.34 ± 2.38	0.0017
Coproccoccus	4.30 ± 2.60	0.73 ± 0.55	0.0007
Finegoldia	0.02 ± 0.02	0.00 ± 0.00	0.0341
Odoribacter	0.19 ± 0.18	0.04 ± 0.06	0.004

	ctrl (%)	**VLR** (%)	**p-value**
Blautia	4.69 ± 2.32	1.26 ± 0.99	0.03
Collinsella	2.04 ± 2.26	0.18 ± 0.25	0.04
Coproccoccus	4.30 ± 2.60	0.64 ± 0.57	0.002
Odoribacter	0.19 ± 0.18	0.05 ± 0.07	0.004

	ctrl (%)	**R** (%)	**p-value**
Collinsella	2.04 ± 2.86	0.49 ± 0.80	0.04
Coproccoccus	4.3 ± 2.6	1.96 ± 2.59	0.002

We next looked at the most abundant genera considering both the time course and the potential effect of the treatments with respect to the control. The expression of the mean relative abundance as a log fold change, with respect to T0, allowed us to appreciate the genera impacted by the different fermentation condition (Figure S2). As already observed, over the 24 hours both control and the different treatments lead to an increase in *Bilophila* and a concomitant decrease of *Roseburia*, *Lachnospira* and *Blautia*. However, a specific pattern of modulation was also observed for other genera. *Holdemania* was increased only upon treatments containing rifaximin. As shown before, *Collinisella* appeared to decrease compared to the control when rifaximin and its combination with lactulose (LR) or VSL#3 (VLR) were fermented. Compared to control, *Parabacteroides* tended to decrease over time in the lactulose + rifaximin (LR) fermentation and by VSL#3 and its combination with the prebiotic (VL) and the antibiotic (VR, VLR). Interestingly already at 5 hours of VLR treatment *Veillonella* seemed to be reduced with respect to the

control. *Bifidobacterium* tended to increase when lactulose alone or VL combination was administered.

2.3.4 Modulation of bacterial composition after treatment

We used fluorescent in situ hybridization coupled with flow cytometry (FISH/FCM) to accurately enumerate different bacterial species and genera. In HS all the treatments, except lactulose, seem to have had little affect on microbial population levels, as shown by the lack of statistically significant variation amongst the treatments. Lactulose showed a small increase in bifidobacteria (data not shown). Differently, CP subjects responded dynamically to the different conditions tested (Figure 6A and B). For the majority of subjects, the use of lactulose or its association with the probiotic resulted in increased numbers of *Bifidobacterium* spp. especially at T10 and T24. A small but significant decrease in the population levels of Enterobacteria was also observed in lactulose and lactulose + VLS#3 fermentations but not under other test conditions.

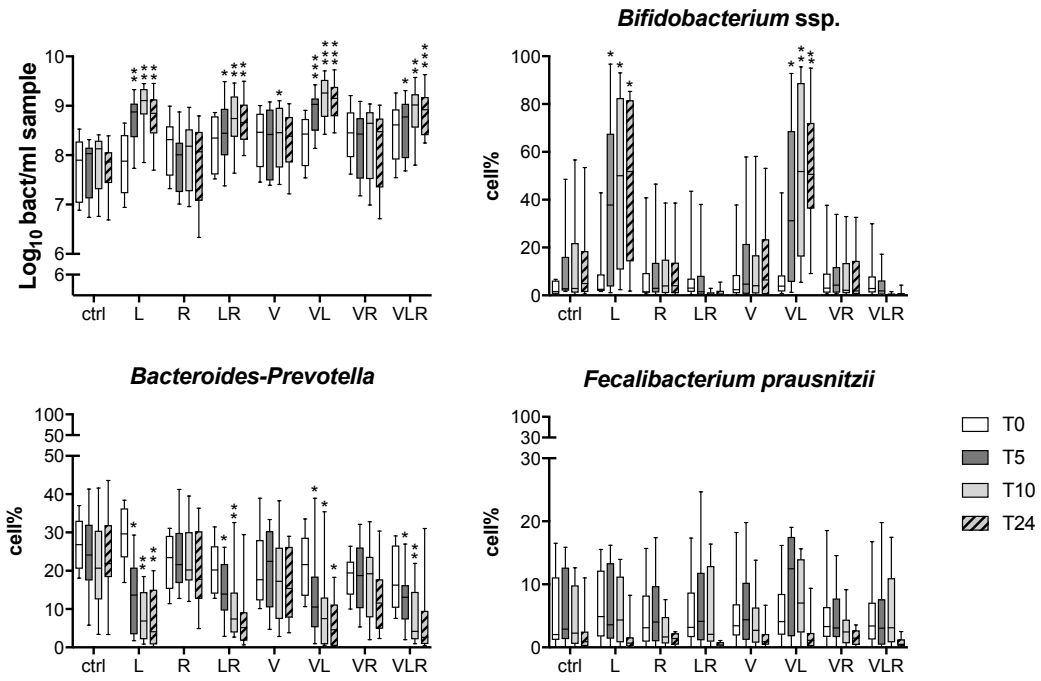

Figure 6A. Bacteria cells enumeration through FISH/FCM, at times 0, 5, 10, and 24 (median-max/min, N = 10) for CP subjects. *p-value≤0.05, **p-value ≤0.01, ***p-value ≤0.001, paired t-test, treatment vs ctrl. ctrl, control; L, Lactulose R, rifaximin; LR, Lactulose + Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VR, VSL#3+Rifaximin; VLR, VSL#3 + lactulose + rifaximin. For the different FISH tested, percentage of positive cells were calculated on gated total bacterial cells.

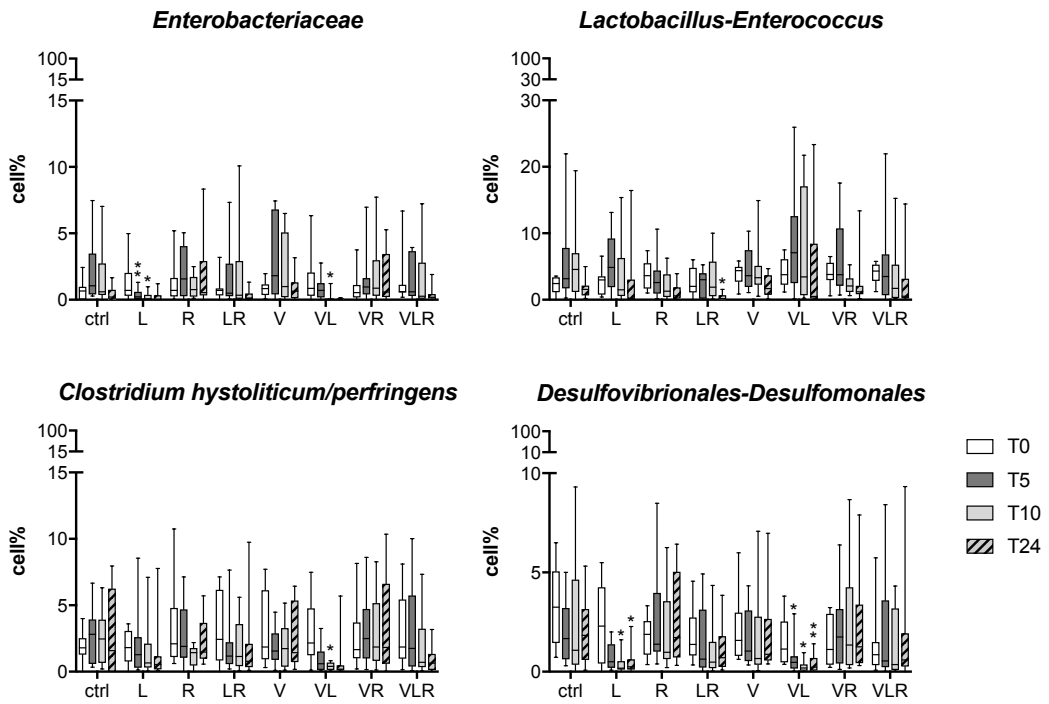

Figure 6B. Bacteria cells enumeration through FISH/FCM, at times 0, 5, 10, and 24 (median-max/min, N = 10) for CP subjects. *p-value≤0.05, **p-value ≤0.01, ***p-value ≤0.001, paired t-test, treatment vs ctrl. ctrl, control; L, Lactulose R, rifaximin; LR, Lactulose + Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VR, VSL#3+Rifaximin; VLR, VSL#3 + lactulose + rifaximin. For the different FISH tested, percentage of positive cells were calculated on gated total bacterial cells.

2.3.5 Modulation of microbial ammonia production

We next investigated if the cirrhotic microbiota modulation by lactulose, rifaximin and VSL#3 treatments induced modifications in the ammonia (NH_3) concentrations produced by the microbiota (Table S9). As reported in Figure 7, absence of treatment showed an increase of the ammonia concentration in a time dependent manner, underlying how the absence of any treatment resulted in a prevalence of ammoniagenic metabolism or ammonia production probably from urea and protein present within the basal medium or inoculum. After microbiota modulatory treatment, NH_3 was particularly reduced at T10, especially when lactulose was combined with rifaximin and VSL#3. Over 24 hours, NH_3 removal was retained when lactulose was combined with rifaximin.

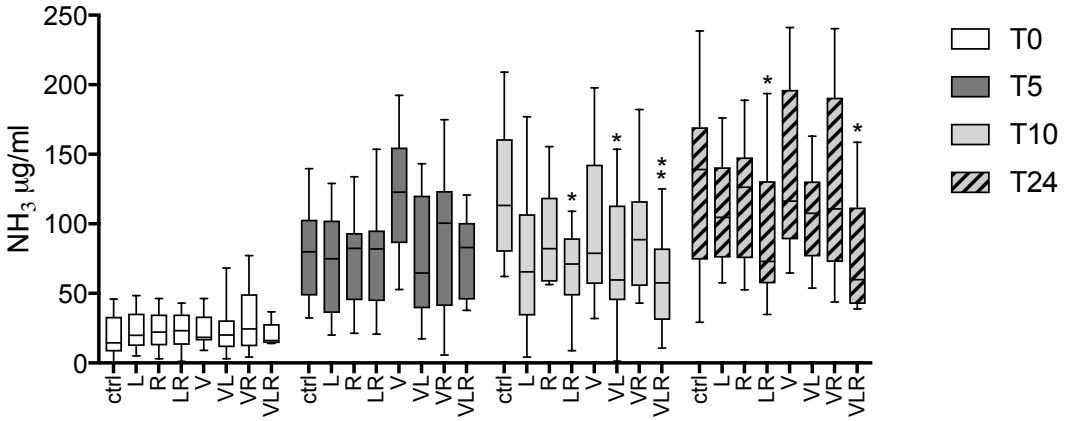

Figure 7. Ammonia level concentration assessed by colorimetric method on the batch culture fermentation supernatant, for each time point considered (median-max/min, N =10, in triplicates) in CP subjects. *p-value≤0.05, paired t-test, treatment vs ctrl. ctrl, control; L, Lactulose R, rifaximin; LR, Lactulose + Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VR, VSL#3+Rifaximin; VLR, VSL#3 + lactulose + rifaximin.

2.3.6 Modulation of microbial SCFA production

Since SCFAs are important for colonic health and may act on neuronal physiology (74–76), we measured the faecal content of SCFAs in our samples by means of GC-MS (Table S10, Figure 8 and Figure S3). We observed that the different treatments induced different SCFA profiles. In general all the treatments determined an increase of SCFA during the 24 h-fermentation period. In particular, acetate content increased over time with respect to the control condition, particularly upon lactulose administration and when the prebiotic was associated with the VSL3 and/or rifaximin after 10 and 24 hours. With respect to the time 0, proprionate and butyrate increased when lactulose was administered alone or in combination with VSL#3. Isobutyrate and valerate tented to decrease significantly after 10 hours especially when rifaximin was in combination with lactulose and/or VSL#3 (Figure S3). No significant modification was observed for isovalerate/2-metyl butyrate (Figure S3).

2.4 Discussion

It is now well recognized that chronic liver diseases such as NAFLD, alcoholic steatohepatitis, NASH and cirrhosis and their extrahepatic complications such as HE, are characterized by gut microbiota dysbiosis, together with alterations in intestinal motility, increased gastric pH and reduced bile acid concentrations in the colon (77–79). Indeed, current clinical treatments are based on manipulation of the gut microbiota, with the principal aim of reducing the production and intestinal absorption of ammonia (41).

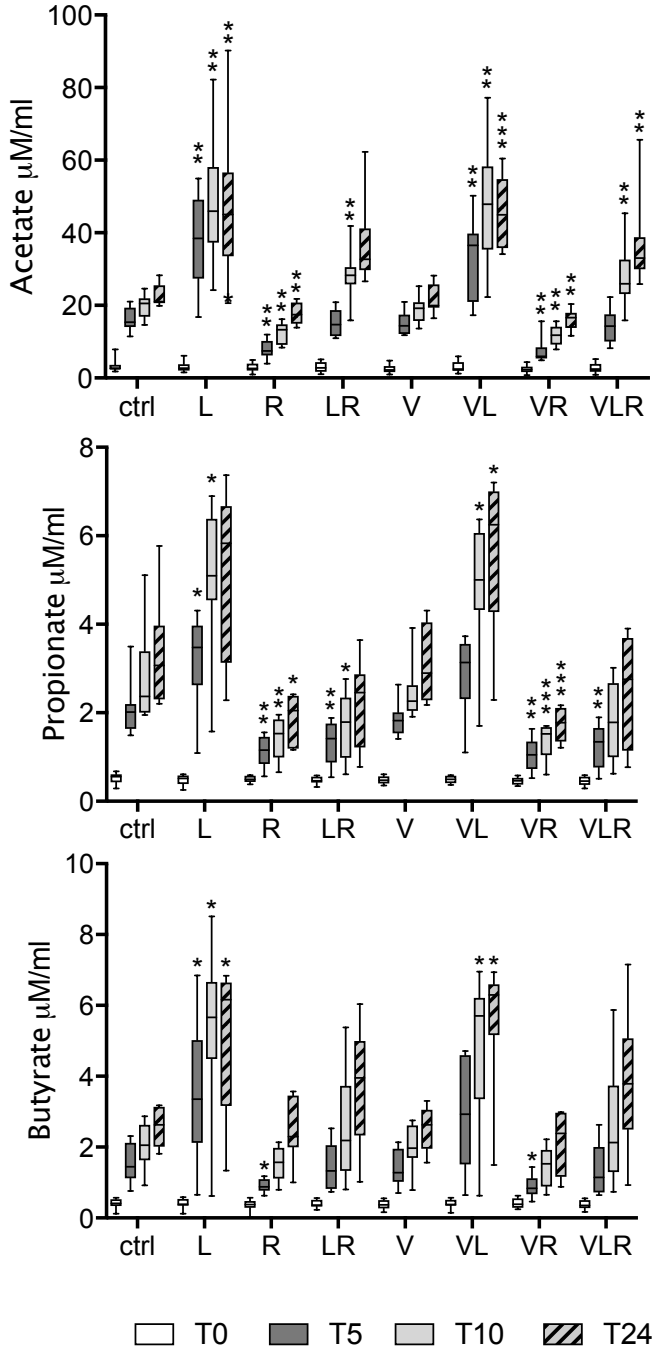

Figure 8. SFCA production at times 0, 5, 10, 24 for Acetate, Propionate and Butyrate (median-max/min, N = 10) in CP subjects. *p-value≤0.05, **p-value ≤0.01, ***p-value ≤0.001, paired t-test, treatment vs ctrl. ctrl, control; L, Lactulose R, rifaximin; LR, Lactulose + Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VR, VSL#3+Rifaximin; VLR, VSL#3 + lactulose + rifaximin.

In this study, we made use of anaerobic pH controlled *in vitro* batch cultures of faecal microbiota from HS and CP to evaluate the modulatory effect of lactulose, VSL#3 and the antibiotic rifaximin. The study was conducted both cross-sectionally – comparing the microbiota response of cirrhotic patients and healthy subjects to treatments at the same time point – and longitudinally, observing the microbiota modulating effect of the pre-, pro- and antibiotic administration over time. Since it has been previously shown that the intestinal microenvironment of cirrhotic subjects without HE is closer to that of healthy subjects (18) it is not surprising that the analysis did not reveal a clear dysbiosis within the microbiota of our cirrhotic patients compared to the healthy subjects at baseline. A trend towards higher relative abundance in Streptococcaceae could be observed in cirrhotic patients in line with previous observations (17,80), although this difference was not statistically significant. Indeed, we found that CP presented a statistically significant higher abundance of *Actinomycetales* lower *Desulfovibrionales* and Desulfovibrionaceae compared to HS. The different aetiologies showed a similar profile, except autoimmune cirrhosis, although this may be due to the fact that there was only one autoimmune CP donor albeit with multiple technical replicates (n = 8). When we moved to specifically characterize the effects of the prebiotic, the probiotic and the antibiotic on the cirrhotic faecal microbiota, we observed a significant increase in the relative abundance of the genus *Bilophila*. *Bilophila* is an intestinal bile resistant pathobiont belonging to the *Proteobacteria* phylum, which is highly represented in NAFLD with respect to healthy group (81,82). However, its overgrowth was potentially due to the bile salt concentration present in the media used for the batch cultures. Increased relative abundance of *Bilophila* was also observed in the control fermentation in which no probiotic, prebiotic or antibiotic was added (Figure S2). Moreover, we observed, independently by the treatment, an increase over time of Erysipelotrichaceae. This bacterial family has been shown to be abundant in choline deficiency-induced fatty liver disease (83), which causes multiple organ dysfunctions. Choline is an important component of our diet, and recently, it was found that choline and phospatidylcholine are converted by the intestinal microbiota to trimethylamine, which is further metabolized to proatherogenic trimethylamine-N-oxide, linking diet and microbiota to cardiovascular disease (84,85). It seems that the different treatments herein used have no effect in reducing the abundance of this taxa within the cirrhotic environment.

Consistent with previous reports the most abundant genera retrieved from the CP microbiota in this study were *Roseburia, Blautia*, *Fecalibacterium, Bifidobacterium, Streptococcus*, *Sutterella, Ruminococcus*, *Parabacteroides* and *Lachnospira* (3,10,86,87). Data showed that the prebiotic, probiotic not antibiotic and their combinations did not change the overall composition of the CP microbiota, but did seem to provide minimal changes on microbiota. In general, however, the different treatments appeared to promote a small reduction of Bacteroidales (Figure 3, Table 3). Increased Bacteroidaceae has been shown as an hallmark of dysbiosis of liver disease (14).

Beyond its bactericidal/bacteriostatic, immuno-modulating and anti-inflammatory activities, a little is known about rifaximin interaction with the gut microbiota (88–92), despite its effect in reducing the risk of HE recurrence and hospitalization rate (93–99). In a previous study rifaximin was shown to induce only a moderate change in the faecal microbiota in HE patients, with a modest reduction of Veillonellaceae abundance and an increase in Eubacteraceae (43). In our study, after the 24 hours fermentation, rifaximin or its association with lactulose or lactulose plus VSL#3 significantly decreased the abundance of Clostridiales, Lachnospiraceae, Vellonellaceae and at genus level, *Blautia* abundance in agreement with a previous study on the mucosal microbiota composition of HE patients supplemented with rifaximin plus lactulose (3). Moreover, rifaximin alone and in combination with lactulose was able to significantly reduce Streptococcaceae relative abundance and concomitantly increase Fusobacteriaceae and Bifidobacteriales (Table 3). As mentioned before, Streptococcaceae were found overabundant in cirrhotic and MHE patients (17) and associated with poor cognitive performance (3). Furthermore, in response to rifaximin we observed a decrease of *Collinsella* with respect to the control (Table 4). *Collinsella* has been recently shown, together with other Firmicutes, such as *Faecalibacterium*, and *Coprobacillus* o be highly represented in mice with NAFLD induced by a high fat diet (100). Indeed, its reduction might be positive in reversing this disease.

The use of VSL#3 in the treatment of HE to date has given contradictory results. In one study, VSL#3 was used to treat cirrhotic patients in a randomized controlled trial and proved effective in preventing HE (11). However, in a second double-blind placebo-controlled study, its supplementation did not show beneficial effects on portal hypertension or decreased hepatic synthetic function (101). Furthermore, a direct link between the ability of VSL#3 to modulate the gut microbiota and amelioration of chronic liver diseases is still missing. Here, we showed that VSL#3 supplementation resulted in an increase in the relative abundance of *Oscillospira* bacteria after 24 hours of batch culture fermentation. Little is known about the role of this bacterial genus within the intestinal tract. However, *Oscillospira* was found positively associated with leanness (102) and reduced in paediatric NASH patients (103). Moreover, a recent study found *Oscillospira* enriched in rats with a lower risk to develop NAFLD (104).

Lactulose has been proven to reduce colonic pH by production of SCFA upon bacterial fermentation, to induce an environment that is both hostile to the survival of urease-producing gut bacteria and facilitates the growth of acid resistant, non-urease-producing species, such as lactobacilli and bifidobacteria. Moreover, the acidification of colonic environment reduces the absorption of ammonia by nonionic diffusion (42). The 16S rRNA community sequencing revealed the ability of lactulose to modulate the gut microbiota in synergy with rifaximin. Moreover, bacterial enumeration by FISH/FCM indicated that the use of lactulose with or without the probiotic VSL#3 induced an increase in bifidobacteria that could account for the concomitant reduction of ammonia levels. From a nutritional point of view, an increased in bifidobacteria has been suggested to enhance

immunity, produce vitamins (folate, B complex), inhibit potential pathogens (105–108) and produce SCFAs. SCFAs, important modulators of host health acting as neuroactive peptides (109), are able to enter the blood and pass the blood brain barrier (110), have anti-inflammatory effects (111) and modulate epigenetic regulation of gene expression (110). SCFA production may also be associated with reduced pH and consequent growth inhibition of pathogenic bacteria. Our results indicate that lactulose, alone or in combination with the probiotic VSL#3 leads to an increase in the SCFA production. Butyrate and acetate are involved in liver lipogenesis and may be involved in regulating fatty acid oxidation and glycogen storage. Propionate acts in the liver as a precursors for de novo gluconeogenesis. Butyrate increase and butyrate-producing bacteria have been suggested to be important in preventing NAFLD and cirrhosis progression (112). Moreover, it was shown that an oral supplementation of sodium butyrate protects mice from inflammation in the liver and consequent cirrhosis development (113).

To summarize, we observed that a prebiotic (lactulose), probiotic (VSL#3) and antibiotic (rifaximin) or their combination, commonly used to treat cirrhosis and HE in clinical practice induce different changes within the gut microbiota of CP under simulated colonic conditions. Although at the community structural level little change was observed, lactulose induced a statistically significant increase in relative abundance and absolute numbers of bifidobacteria. However, significant changes were observed from different treatment at the metabolic level. Lactulose, or lactulose combined with antibiotic or antibiotic plus probiotic, consistently lowered ammonia production and increased production of SCFA. This shift in metabolite production is indicative of carbohydrate fermentation that could also significantly increased consumption or conversion of ammonia and other nitrogenous compounds in bacterial biomass. In either case, reduced ammonia concentrations and increased concentration of SCFA are consistent with improved gut health and reduced risk of HE.

Several directions of research are opened by this study: on the one hand future investigations should assess the molecular pathways that are involved in the modulation of gut microbiota and its metabolic reprogramming and on the other hand translational studies should assess the clinical potential of these *in vitro* observation. Moreover, a deep analysis of patients' response to treatment could identify the microbiota profile of responders and non responders helping in defining personalized therapies.

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Supplementary Figures

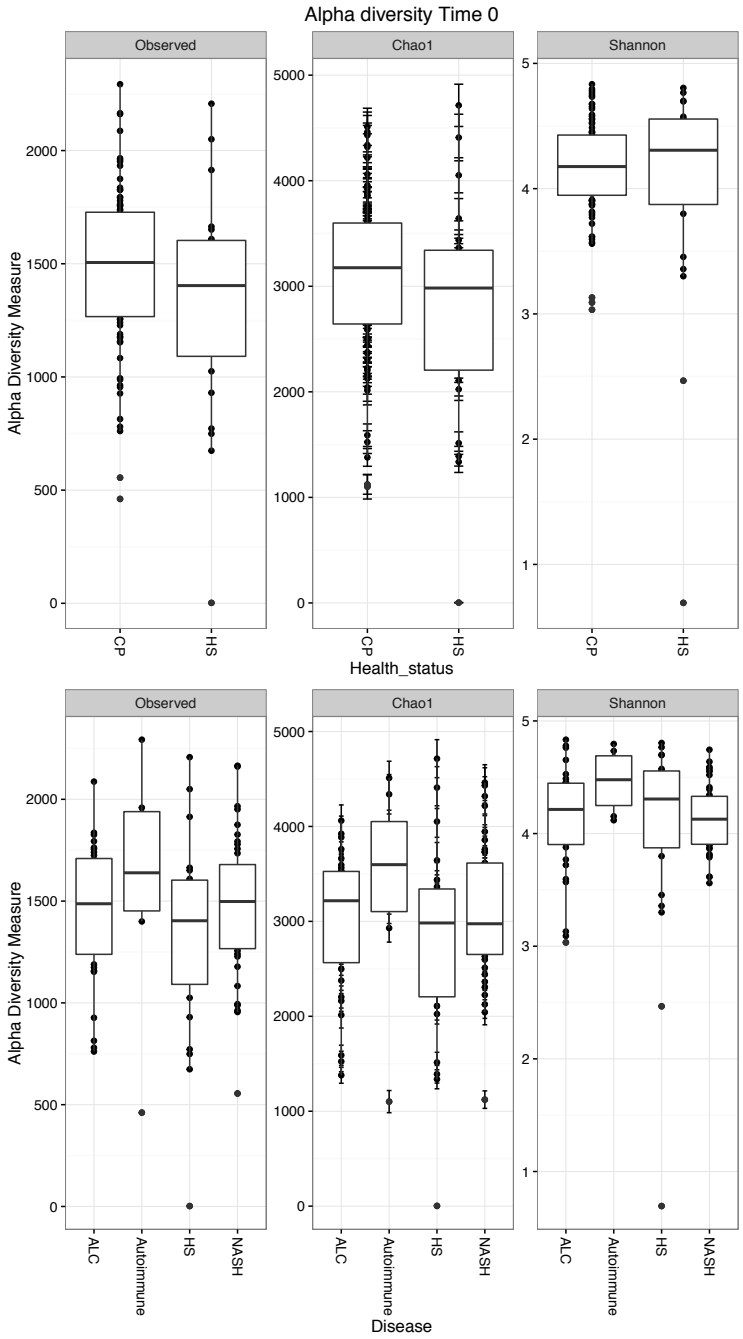


Figure S1. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for CPvs.HS and also respect etiology at T0. The body of the box plot represents the first and third quartiles of the distribution and the median. The whiskers extend from the quartiles to the last datapoint within 1.5× IQR with outliers beyond represented as dots. ALC, alcohol liver cirrhosis; NASH, nonalcoholic steatohepatitis.

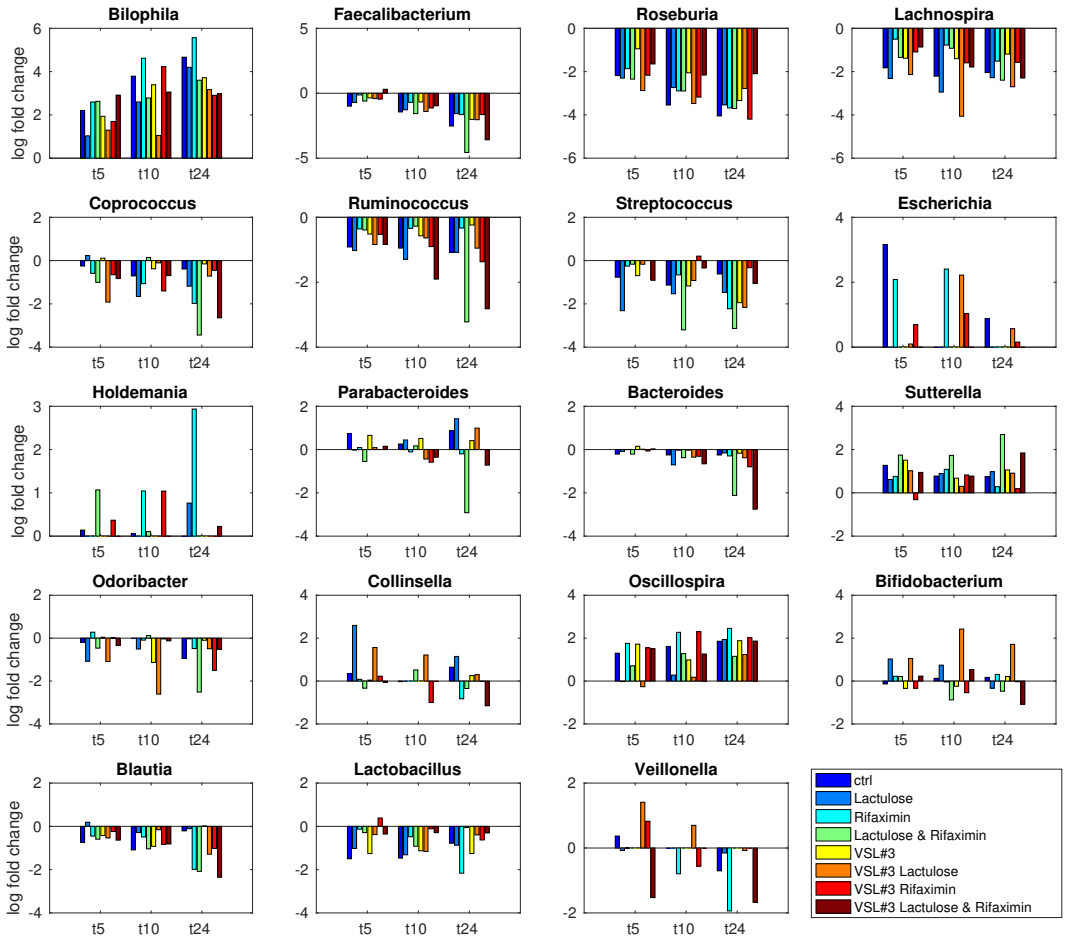


Figure S2. Log fold change trend for the main genera in CP. Data are represented as mean relative abundance log ratio of the different condition at T5, T10, T24 respect to T0

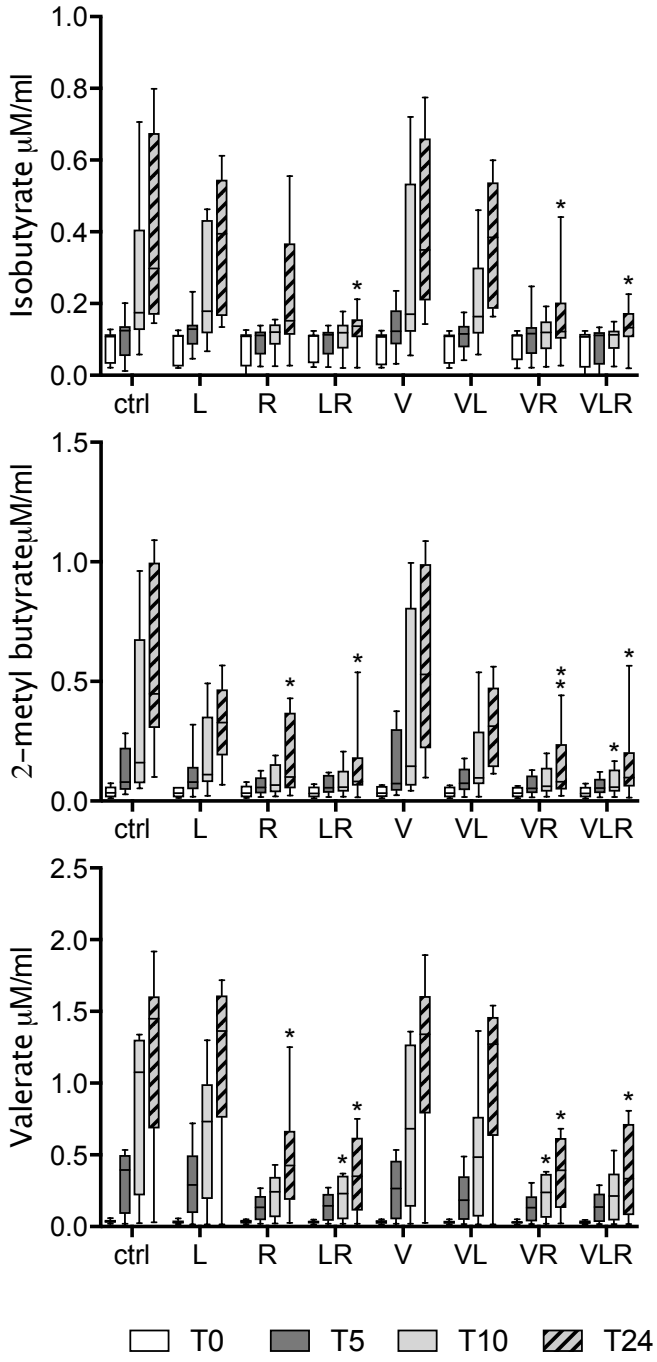


Figure S3. SFCA production at times 0, 5, 10 and 24 for Isobutyrate, 2-methyl butyl isovalerate and Valerate. (median-max/min, N = 10). *p-value≤0.05, **p-value ≤0.01, ***p-value ≤0.001, paired t-test, treatment vs ctrl. ctrl, control; L, Lactulose R, rifaximin; LR, Lactulose + Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VR, VSL#3+Rifaximin; VLR, VSL#3 + lactulose + rifaximin.

Supplementary Tables

Table S1. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for Healthy subjects (HS), Cirrhotic patients (CP) and CP subgroups according to the aetiologies s at the baseline. ALC, alcohol cirrhosis, AI, autoimmune cirrhosis, NASH, non-alcoholic stehatosis.

Populations (T0)	Metric	p-value
CP vs. HS	Observed	0.1437
	Chao1	0.1709
	Shannon	0,8440
HS vs. Ai	Observed	0.3457
	Chao1	0.2873
	Shannon	0.3205
HS vs. ALC	Observed	0.3457
	Chao1	0.4229
	Shannon	0.8502
HS vs. NASH	Observed	0.3457
	Chao1	0.3200
	Shannon	0.6897
Ai vs. ALC	Observed	0.3457
	Chao1	0.2873
	Shannon	0.2266
Ai vs. NASH	Observed	0.3457
	Chao1	0.2873
	Shannon	0.0681
ALC vs. NASH	Observed	0.7168
	Chao1	0.6874
	Shannon	0.6897

Table S2. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity, for for Healthy subjects (HS), Cirrhotic patients (CP) and CP subgroups according to the aetiologies s at the baseline.

Populations (T0)	Metric	*F*	R^2	p-value
CP vs. HS	Unweighted UniFrac	31.938	0,03036	0,035
	Weighted UniFrac	14.251	0,01378	*0,008*
	Bray-Curtis	26.416	0,02524	*0,001*

Health status	Unweighted UniFrac	0.6254	0.04361	0.896
	Weighted UniFrac	0.87729	0.06012	0.999
	Bray-Curtis	0.84347	0.05794	0.881

Table S3. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for HS subjects, overtime for for each condition considered. No statistically significant differences have been found. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

		Time point vs time point					
		T0vsT5	**T0vsT10**	**T0vsT24**	**T5vsT10**	**T5vsT24**	**T10vsT24**
Treatment	**Metric**	**p-value***	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
ctrl	Observed	1	1	1	1	1	1
	Chao1	1	1	1	1	1	1
	Shannon	1	1	1	1	1	1
L	Observed	0.8	0.8	1	0.84	0.84	0.8
	Chao1	1	0.8	1	1	0.8	0.8
	Shannon	0.4	0.4	1	0.48	0.4	0.48
R	Observed	1	1	1	1	1	1
	Chao1	0.7	0.7	0.7	0.7	0.6	0.7
	Shannon	1	1	1	1	0.6	0.6
LR	Observed	1	0.8	1	0.8	0.8	1
	Chao1	0.84	0.8	0.84	0.8	0.8	1
	Shannon	1	0.3	0.3	0.4	0.6	1
V	Observed	0.8	1	1	0.8	1	0.8
	Chao1	1	1	1	1	1	1
	Shannon	0.6	0.6	0.6	1	1	0.6
VL	Observed	1	1	1	1	1	1
	Chao1	1	1	0.8	1	0.8	0.8
	Shannon	0.7	0.48	0.4	0.48	0.3	0.3
VR	Observed	0.7	0.7	0.7	0.7	0.7	0.7
	Chao1	1	1	1	1	1	1
	Shannon	1	1	1	1	1	1
VLR	Observed	1	0.8	0.8	0.8	1	1
	Chao1	1	0.84	0.84	0.84	0.84	0.84
	Shannon	1	1	1	1	1	1

*FDR corrected p-values

Table S4. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity, for HS subjects, overtime for for each condition considered. No statistically significant differences have been found. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

Treatment	Metric	*F*	R^2	p-value*
ctrl	Unweighted UniFrac	1	0.2200	0.611
	Weighted UniFrac	1	0.2603	0.821
	Bray-Curtis	0.86992	0.2459	0.739
L	Unweighted UniFrac	10083	0.2743	0.488
	Weighted UniFrac	0.98807	0.2703	0.527
	Bray-Curtis	11.612	0.3033	0.2
LR	Unweighted UniFrac	0.72497	0.2137	0.857
	Weighted UniFrac	0.94057	0.2607	0.738
	Bray-Curtis	1	0.2328	0.807
R	Unweighted UniFrac	16.351	0.3801	0.153
	Weighted UniFrac	1	0.2681	0.52
	Bray-Curtis	11.797	0.3067	0.267
V	Unweighted UniFrac	16.753	0.3858	0.127
	Weighted UniFrac	1	0.2615	0.796
	Bray-Curtis	10.118	0.2750	0.46
VL	Unweighted UniFrac	11.375	0.2990	0.333
	Weighted UniFrac	1	0.2504	0.901
	Bray-Curtis	1	0.2686	0.506
VR	Unweighted UniFrac	1	0.2353	0.52
	Weighted UniFrac	1	0.2424	0.99
	Bray-Curtis	1	0.1947	0.973
VLR	Unweighted UniFrac	10858	0.2893	0.378
	Weighted UniFrac	0.94017	0.2606	0.715
	Bray-Curtis	1	0.2137	0.802

*Bonferroni corrected p-values

Table S5. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for CP subjects, overtime for each condition considered. Significant differences are reported in italic. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

		Time point vs time point					
		T0vsT5	**T0vsT10**	**T0vsT24**	**T5vsT10**	**T5vsT24**	**T10vsT24**
Treatment	**Metric**	**p-value***	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
ctrl	Observed	0.4359	*0.0006*	*0.0006*	*0.0030*	0.0058	0.4232
	Chao1	0.7394	*0.0012*	*0.0030*	*0.0030*	*0.0220*	0.2611
	Shannon	0.9705	0.3806	0.9551	0.3806	0.9551	0.3806
L	Observed	0.7054	0.5290	0.5290	0.5290	0.7054	0.5290
	Chao1	0.7219	0.7219	0.7394	0.7219	0.7394	0.7219
	Shannon	0.6346	0.4294	0.9118	0.6346	0.4351	0.4294
R	Observed	0.6842	0.3712	0.0881	0.6346	0.3712	0.2258
	Chao1	0.7394	0.7394	0.0690	0.7394	0.4949	0.2258
	Shannon	0.4198	0.5230	0.2102	0.9705	0.2102	0.2102
LR	Observed	0.2855	0.1278	*0.0035*	0.5204	0.1127	0.4132
	Chao1	0.2460	0.3263	*0.0102*	0.6842	*0.0339*	0.3688
	Shannon	0.1784	0.3358	*0.0011*	0.6305	*0.0173*	0.2427
V	Observed	0.6945	0.6945	0.6945	0.6945	1.0000	0.6945
	Chao1	0.7566	0.7566	0.7566	0.7566	0.9705	0.7566
	Shannon	0.5775	0.2460	0.6842	0.4198	0.2460	0.2460
VL	Observed	0.1049	0.1049	0.1049	0.9705	0.9705	0.9705
	Chao1	0.0865	0.0865	0.0865	0.9705	0.9705	0.9705
	Shannon	0.3146	0.3310	0.3310	0.5775	0.5290	0.8534
VR	Observed	0.6842	0.1890	0.3310	0.1890	0.4198	0.5394
	Chao1	0.4813	0.0945	*0.0019*	0.3358	*0.0045*	*0.0465*
	Shannon	0.7394	0.5230	*0.0032*	0.5230	*0.0019*	*0.0371*
VLR	Observed	0.1718	0.0532	*0.0019*	0.4359	*0.0022*	*0.0294*
	Chao1	0.7959	0.0864	0.2863	0.0864	0.3263	0.7959
	Shannon	0.8534	0.8534	0.8534	0.8534	0.8534	0.8534

*FDR corrected p-values

Table S6. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity, for CP subjects, overtime for for each condition considered. Significant differences are reported in italic. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

Treatment	Metric	*F*	R^2	p-value*
ctrl	Unweighted UniFrac	1	0.0647	0.569
	Weighted UniFrac	10835	0.0822	0.11
	Bray-Curtis	1.604	0.1179	0.006
L	Unweighted UniFrac	0.6035	*0.0478*	0.847
	Weighted UniFrac	0.9742	0.0759	0.589
	Bray-Curtis	11.486	0.0873	0.206
R	Unweighted UniFrac	0.5995	*0.0475*	0.811
	Weighted UniFrac	0.9971	0.0767	0.469
	Bray-Curtis	12.248	0.0926	0.126
LR	Unweighted UniFrac	17.325	0.1261	0.1
	Weighted UniFrac	12.642	0.0953	*0.001*
	Bray-Curtis	23.195	0.1619	*0.002*
V	Unweighted UniFrac	13.939	0.1040	0.156
	Weighted UniFrac	10.461	0.0801	0.265
	Bray-Curtis	13.392	0.1003	0.053
VL	Unweighted UniFrac	16.076	0.1181	0.12
	Weighted UniFrac	10.931	0.0834	0.071
	Bray-Curtis	12.101	0.0916	0.144
VR	Unweighted UniFrac	1	*0.0441*	0.889
	Weighted UniFrac	1.238	0.0935	*0.003*
	Bray-Curtis	1.908	0.1371	*0.001*
VLR	Unweighted UniFrac	0.6122	*0.0485*	0.784
	Weighted UniFrac	0.9952	0.0765	0.489
	Bray-Curtis	11.052	0.0843	0.261

*Bonferroni corrected p-values

Table S7. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index, for CP subjects, between considered treatments. Significant values are reported in italic. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

		Time point			
		T0	T5	T10	T24
Treatment vs treatment	**Metric**	**p-value***	**p-value**	**p-value**	**p-value**
ctrl vs L	Observed	0.9579	0.8095	0.8466	0.6474
	Chao1	0.9808	0.6245	0.8626	0.5224
	Shannon	0.9456	0.9872	0.9705	0.8914
ctrl vs R	Observed	0.9579	0.9337	0.2180	0.9456
	Chao1	0.9808	0.9558	0.1916	0.6170
	Shannon	0.9456	0.9872	0.7337	0.1505
ctrl vs LR	Observed	0.9579	0.8095	0.8626	0.2911
	Chao1	0.9808	0.6245	0.7596	0.0738
	Shannon	0.9456	0.6769	0.9292	*0.0014*
ctrl vs V	Observed	0.9579	0.8095	0.9456	10
	Chao1	0.9808	0.9558	0.9705	0.8254
	Shannon	0.9456	0.9872	0.9003	0.9705
ctrl vs VL	Observed	0.9579	0.8095	0.4305	0.6474
	Chao1	0.9808	0.6888	0.5010	0.6170
	Shannon	0.9456	0.6769	0.9705	0.8025
ctrl vs VR	Observed	0.9579	0.8095	0.8626	0.9456
	Chao1	0.9808	0.6245	0.8626	0.6170
	Shannon	0.9456	0.6769	0.8710	0.5188
ctrl vs VLR	Observed	0.9579	0.8095	0.8229	*0.0291*
	Chao1	0.9808	0.6245	0.7596	*0.0292*
	Shannon	0.8326	0.6769	0.9705	*0.0014*
ctrl vs R	Observed	0.9579	0.9337	0.6092	0.6474
	Chao1	0.9808	0.9558	0.7596	0.5512
	Shannon	0.9456	0.6769	0.7337	0.0932
L vs LR	Observed	0.9579	0.9337	0.8626	0.0843
	Chao1	0.9808	0.9558	0.8626	0.0689
	Shannon	0.9456	0.9872	0.9705	*0.0027*
L vs V	Observed	1	1	0.8626	0.7404
	Chao1	1	0.9558	0.8626	0.6170
	Shannon	0.9456	10	0.7337	0.9705
L vs VL	Observed	1	1	0.6092	0.9286
	Chao1	1	0.9558	0.6769	0.6170

	Shannon	0.9456	1	0.9705	0.6114
L vs VR	Observed	1	1	0.8626	0.6474
	Chao1	1	0.9705	0.8626	0.3310
	Shannon	0.9456	1	0.7337	0.6738
L vs VLR	Observed	0.9858	0.9337	0.8626	*0.0136*
	Chao1	1	0.9558	0.9191	*0.0292*
	Shannon	0.8703	1	0.9705	*0.0014*
R vs LR	Observed	1	0.9337	0.2180	0.3082
	Chao1	1	0.9558	0.5010	0.1052
	Shannon	0.9456	0.6769	0.7337	*0.0014*
R vs V	Observed	0.9579	0.9337	0.4205	0.9456
	Chao1	0.9808	0.9558	0.4906	0.9118
	Shannon	0.9456	0.9872	0.7337	0.5188
R vs VL	Observed	0.9579	0.9337	0.2422	0.6780
	Chao1	1	0.9558	0.2482	0.6170
	Shannon	0.9456	0.6769	0.7337	0.4061
R vs VR	Observed	0.9579	0.9337	0.2180	0.9286
	Chao1	0.9808	0.9558	0.2482	0.6170
	Shannon	0.9456	0.6769	0.7337	0.9705
R vs VLR	Observed	0.9579	0.9337	0.2180	*0.0291*
	Chao1	0.9808	0.9558	0.2482	*0.0383*
	Shannon	0.8326	0.6769	0.7337	*0.0027*
LR vs V	Observed	0.9579	10	0.9456	0.4333
	Chao1	0.9808	0.9558	0.8626	0.1370
	Shannon	0.9456	0.9872	0.7337	*0.0020*
LR vs VL	Observed	0.9579	0.9337	0.8626	0.1989
	Chao1	1	0.9558	0.9705	0.1052
	Shannon	0.8703		0.9705	*0.0066*
LR vs VR	Observed	0.9579	0.9337	0.8626	0.6474
	Chao1	0.9808	0.9558	0.8626	0.2608
	Shannon	0.9456	1	0.9705	*0.0027*
LR vs VLR	Observed	0.9579	0.9337	1	0.1578
	Chao1	0.9808	0.9558	0.8626	0.2426
	Shannon	0.8703	1	0.9579	0.8914
V vs VL	Observed	0.9579	1	0.8018	0.9286
	Chao1	0.9808	0.9558	0.7596	0.8254
	Shannon	0.9456	1	0.7337	0.7051
V vs VR	Observed	1	0.9337	0.9456	0.9286
	Chao1	1	0.9558	0.8914	0.6482
	Shannon	0.9705	1	0.7337	0.6738

V vs VLR	Observed		0.9337	0.8626	*0.0291*
	Chao1	1	0.9705	0.8626	*0.0363*
	Shannon	0.9456	1	0.7337	*0.0018*
VL vs VR	Observed	0.9579	1	0.3512	0.7404
	Chao1	0.9808	0.9558	0.4214	0.6170
	Shannon	0.9456	1	0.7337	0.8914
VL vs VLR	Observed	0.9579	0.9337	0.8626	*0.0291*
	Chao1	0.9808	0.9558	0.8626	*0.0364*
	Shannon	0.4113	1	0.8710	*0.0027*
VR vs VLR	Observed	1	0.9337	0.8626	*0.0319*
	Chao1	1	0.9705	0.8626	0.0536
	Shannon	0.8703	1	0.7337	*0.0027*

*FDR corrected p-values

Table S8. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity, for CP subjects, between the considered treatments. Significant valies are reported in italic. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

Time point	Metric	*F*	R^2	p-value*
T0	Unweighted UniFrac	0	0.04422	0.981
	Weighted UniFrac	0.87846	0.07869	0.995
	Bray-Curtis	1	0.07683	0.841
T5	Unweighted UniFrac	0.5284	0.04886	0.961
	Weighted UniFrac	0.9216	0.08223	0.974
	Bray-Curtis	0.87487	0.07839	0.827
T10	Unweighted UniFrac	0.7031	0.06398	0.843
	Weighted UniFrac	10.158	0.08988	0.33
	Bray-Curtis	12.745	0.11025	*0.027*
T24	Unweighted UniFrac	1.063	0.09367	0.389
	Weighted UniFrac	1.185	0.10331	*0.002*
	Bray-Curtis	17.686	0.14672	*0.001*

*Bonferroni corrected p-values

Table S9. Ammonia level in CP batch cultures. Values are presented as median values (µg/ml). ctrl, control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

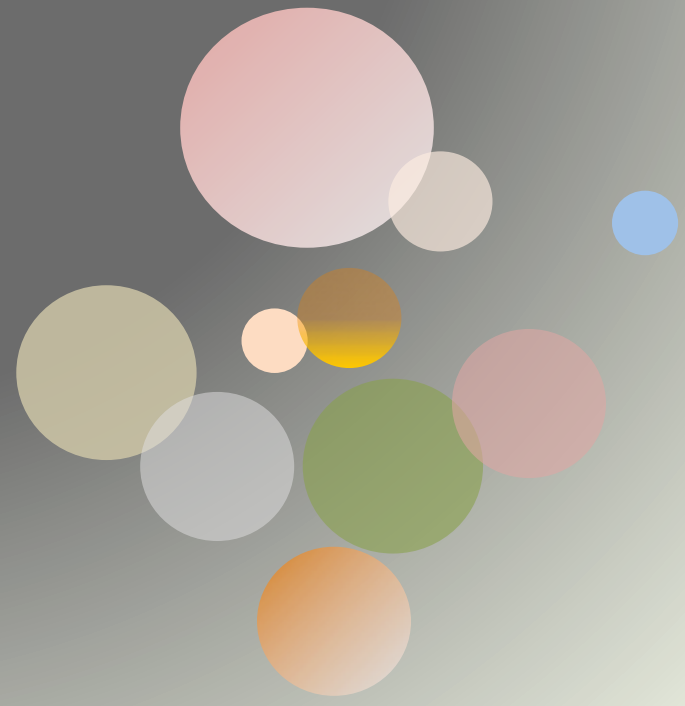
	Time points			
Treatment	**0**	**5**	**10**	**24**
ctrl	14.58	79.84	113.26	139.06
L	19.90	74.92	65.59	104.62
L+R	23.32	81.99	71.17	73.10
R	22.08	82.43	82.10	126.27
V	18.37	122.88	78.93	149.52
V+L	20.04	64.72	59.62	108.67
V+L+R	16.11	83.09	57.50**	59.95
V+R	24.49	100.47	88.70*	140.70

Table S10. SCFA content in CP batch cultures. The different SCFAs are presented as median values (µmol/g). ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

Treatment	Time point	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate/2MeBut	Valerate	total SCFA
ctrl	0	2.53	0.56	0.11	0.41	0.03	0.03	3.68
	5	15.38	2.01	0.12	1.45	0.08	0.39	19.44
	10	20.54	2.37	0.17	2.05	0.16	1.08	26.37
	24	21.08	3.07	0.30	2.62	0.45	1.45	28.97
L	0	2.72	0.52	0.11	0.38	0.03	0.03	3.79
	5	38.50	3.48	0.13	3.36	0.08	0.29	45.83
	10	45.97	5.10	0.18	5.66	0.11	0.73	57.76
	24	45.04	5.83	0.39	6.16	0.33	1.36	59.11
L+R	0	2.79	0.47	0.11	0.36	0.03	0.03	3.79
	5	14.72	1.42	0.11	1.33	0.06	0.14	17.78
	10	28.30	1.79	0.12	2.18	0.06	0.23	32.69
	24	32.70	2.46	0.14	3.94	0.08	0.35	39.67
R	0	2.61	0.49	0.11	0.38	0.03	0.04	3.66
	5	7.50	1.16	0.11	0.88	0.06	0.13	9.83
	10	13.29	1.53	0.12	1.57	0.07	0.24	16.82
	24	17.67	2.13	0.15	2.34	0.10	0.43	22.81
V	0	2.21	0.48	0.11	0.37	0.03	0.03	3.23
	5	14.37	1.82	0.12	1.28	0.07	0.27	17.94
	10	19.21	2.26	0.17	1.97	0.15	0.68	24.45
	24	19.97	2.90	0.35	2.62	0.53	1.34	27.72

V+L	0	2.50	0.50	0.11	0.37	0.03	0.03	3.53
	5	36.50	3.14	0.12	2.93	0.07	0.19	42.94
	10	47.90	5.01	0.16	5.70	0.10	0.48	59.35
	24	44.93	6.25	0.38	6.29	0.31	1.27	59.44
V+L+R	0	2.45	0.46	0.11	0.36	0.03	0.03	3.43
	5	14.29	1.35	0.11	1.15	0.05	0.14	17.08
	10	25.98	1.78	0.11	2.13	0.06	0.21	30.27
	24	33.07	2.75	0.14	3.78	0.10	0.33	40.18
V+R	0	2.28	0.46	0.11	0.39	0.03	0.03	3.31
	5	5.94	1.05	0.12	0.84	0.05	0.13	8.14
	10	11.81	1.53	0.12	1.53	0.06	0.24	15.28
	24	16.64	1.78	0.12	2.38	0.08	0.39	21.40

Chapter 3



Effect of VSL#3 treatment in paediatric patients and young adults affected by Minimal Hepatic Encephalopathy from portal hypertension: a pilot intervention study

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Authors' contributions:
KMT, PA and *LDA* conceived the idea
AM performed DNA extraction, PCR amplification, SCFA profiling, bioinformatics data analysis and wrote the manuscript
SG enrolled the patients, collected samples and performed the clinical assessment
MP performed library preparation and 16S rRNA sequencing
KMT revised the manuscript

-Paper in preparation-

Keywords

Portal hypertension, probiotic, VSL#3, gut microbiota, minimal hepatic encephalopathy, liver disease

Abstract

Many liver and vascular diseases cause portal hypertension in children, which may give rise to severe and life-threatening complications, including hepatic encephalopathy. The effective prevention and management of portal hypertension and its complications, therefore, are important goals for improving the quality of life of affected children. Restoration and modulation of intestinal microbiota using probiotics has potential in treating symptoms associated with acute liver diseases and chronic liver failure. We assessed the efficacy of the probiotic VSL#3 in modulating the gut microbiota and reducing the severity of portal vein hypertension and Minimal Hepatic Encephalopathy in paediatric and young adult patients with liver disease. We performed a double-blind trial by assigning randomly patients to groups given the probiotic preparation or placebo daily for 3 months. Fecal samples were collected at the beginning of the experiment and after the treatment and 16S rRNA gene metataxonomic analysis was performed. VSL#3 supplementation resulted in a trend toward improved cognitive function but not change in the gut microbiota was observed. High inter-individual variation in gut microbiota was observed. The study was confounded by the low study sample size and the different underlying aetiologies of the portal vein hypertension patients. Thus, a larger study with a more potent stratification for different underlying liver disease is needed to prove the link between gut microbiota changes in terms of community structure and metabolism and the efficacy of VSL#3 in ameliorating the disease condition.

3.1 Introduction

The intestinal microbiota plays an important role in health and disease. Alteration in its healthy homeostasis, dysbiosis and modification of intestinal permeability leading to bacterial translocation may result in the development of numerous liver disorders or worsening of hepatic disorders, such as cirrhosis, portal hypertension, hepatic encephalopathy (HE) and acute-on-chronic-liver failure (1,2). Antibiotics appear to effectively reduce the impact of these complications of liver diseases mainly through their effect on intestinal microbiota (1,2). However, due to the increasing drug resistance, alternatives to antibiotics are now considered for the prevention of bacterial translocation and its consequences (3). Such alternatives include prebiotics and probiotics which effectively modulate the ecology of the gut microbiota (4–6).

Generally defined as “live microorganisms that produce a beneficial effect to the host when administered in an adequate amount (7)”, interest towards probiotics has grown in recent years, partly because their administration is safe, inexpensive and they represent a noninvasive approach to prevent and treat a variety of diseases, including hepatic disorders (3,8). In the context of liver diseases, some evidence supports probiotic efficacy for (i)

changing gut metabolism; (ii) reducing ammonia in the portal blood (decreasing bacterial urease activity, intestinal permeability, ammonia absorption by lowering pH and improving nutritional status of gut epithelium); (iii) reducing hepatic inflammation and oxidative stress; (iv) reducing the absorption of other toxins such as indoles, oxindoles, phenols and mercaptans (9–11).

Several studies in animal models of non-alcoholic fatty liver disease (NAFLD) have reported beneficial effects of certain probiotics on liver damage, such as reduction in hepatic total fatty acid content and liver inflammation as well as an improvement in hepatic insulin resistance (9–11). However, most studies evaluating probiotics in experimental models of cirrhosis and portal hypertension gave contrasting results. No benefits on intestinal microbiota or bacterial translocation have been observed with *Lactobacillus rhamnosus* GG (12) and *Lactobacillus johnsonii* La1 (13), or with *Lactobacillus acidophilus* and *Lactobacillus rhamosus* GG in rats with portal vein ligation (14). Nevertheless, several human intervention studies, mainly in the field of HE, have provided supportive evidence for the efficacy of certain probiotics in the improvement of Minimal HE (MHE), in the prevention of HE recurrence and prophylaxis of Overt HE (OHE). In these studies the probiotics appeared to be acting by reducing plasma ammonia concentrations and endotoxemia levels (15–20) and improving cognitive function as measured by neuropsychiatric tests (21). Use of *Escherichia coli* Nissle strain was also reported to result in improvement in liver function, as measured by Child- Pugh score, and also reducing blood endotoxin levels (22). Patients with cirrhosis and minimal HE treated for 1 month with a probiotic combination (*Pediococcus pentoseceus* 5–33:3, *Leuconostoc mesenteroides* 32–77:1, *Lactobacillus paracasei* spp. *paracasei* 19 and *Lb. plantarum* 2592) administered with a fermentable fiber, significantly increased the fecal content of non-urease-producing lactobacilli with a decrease in *Escherichia coli* fecal concentrations, decrease in blood endotoxemia, and an improvement in liver function and cognitive tests (23).

The most studied probiotic in treating chronic liver diseases and their complication is VSL#3. VSL#3 is a multispecies probiotic containing 8 bacterial strains, *Lb. paracasei*, *Lb. plantarum*, *Lb. acidophilus*, *Lb. delbrueckii* spp. *bulgaricus*, *Bifidobacterium longum*, *B. breve*, *B. infantis* and *Streptococcus salivarius* spp. *thermophilus* (24). This mixture has been observed to decrease fibrosis in an experimental model of non-alcoholic steatohepatitis in mice (25), to prevent endothelial dysfunction in the mesenteric artery (26) and to reduce bacterial translocation, pro-inflammatory state and increase tight junctions and intestinal integrity in cirrhotic rats (26–29) . In patients with different liver diseases VSL#3 has been shown to improve liver function tests, pro-inflammatory cytokines and oxidative damage (29). Cirrhotic and decompensated cirrhotic subjects benefit of VSL#3 treatment due to the associated improvement in hepatic and systemic haemodynamics as well as in portal hypertension (30–32). Moreover, preventive effects of probiotic intervention in patients with liver cirrhosis, who had not experienced OHE, showed that VSL#3 may be useful in the prevention of HE and in the treatment of minimal

HE (33,34). As these trials concluded the bulk of evidence favours the use of certain probiotics for ameliorating MHE disease symptoms by modulation of gut microbiota metabolism and ammonia levels (35). To note, the potentiality of probiotics supplementation, namely *Lb. rhamnosus* strain GG (36) and VSL#3 (37), vs. placebo, to improve transaminase level and reduce plasma LPS level has been evidenced also in paediatric patients with pre-cirrhotic biopsy-proved NAFLD state.

In paediatric age, acute liver diseases and chronic liver diseases - from NAFLD to portal hypertension to acute liver failure - represents an increasing issue, where diagnosis and management are a challenge. Cases of hepatic encephalopathy are increasing (38,39). However, there is little data associating probiotic use with improvements in portal vein hypertension or HE and what data does exist, has been generated from studies in adults. Children with primary extrahepatic portal vein thrombosis have portal-systemic shunting, which may lead to disturbed neurocognitive function similar to portal-systemic encephalopathy (PSE) and MHE seen with chronic liver disease and cirrhosis in adults. Bacterial translocation plays a role in increasing portal pressure by exacerbating the hyperdynamic circulatory state and increasing hepatic vascular resistance. Moreover the induced portal circulation shunt leads to reduced blood circulation in the liver and consequently passage of gut-derived un-detoxified compounds in the main circulation, including ammonia, the principal toxic molecules involved in triggering HE onset (40). Previously, a 6-week administration of VSL#3 resulted in reductions of the hepatic vascular resistance, as well as in the improvement of systemic haemodynamics (30,41). Data in this area are conflicting. Two studies in adult patients with compensated or decompensated cirrhosis utilizing VSL#3 for 2 months did not reduce hepatic venous pressure gradient and no changes in gut microbiota were observed as measured by terminal restriction fragment length polymorphism (TRFLP). However the authors did report reductions in plasma endotoxemia and inflammatory cytokines (31,42).

Here, we present a pilot intervention study in paediatric and young adults afflicted by portal vein hypertension and manifesting symptoms of MHE. Intervention was with VSL#3 or placebo for 3 months. 16S rRNA sequencing has been performed on faecal samples before and after the treatment. Data were correlated with cognitive function improvement measured by neuropsychiatric tests. The overall aim was to study the effect of VSL#3 in ameliorating impaired cognitive function and patients quality of life by modulating the gut microbiota and reducing the ammonia level.

3.2 Material and Methods

3.2.1 Patients enrollment and intervention study

The clinical trial (ClinicalTrials.gov Identifier: NCT01798329) expected the enrolment of 50 patients with clinical diagnosis of prehepatic portal hypertension and developing Minimal Hepatic Encephalopathy. During the duration of the study we were able to recruite 18 patients (median age 10, range 4-18). In particular subjects were affected by portal vein hypertension [cavernome portal hypertension (n=2), portal

thrombosis (n=3), congenital hepatic fibrosis (n=1), biliary atresia (n=10) and sclerosis cholangitis (n=1)]. All subjects of this study were under a Mediterranean-based diet and no antibiotics, probiotics or prebiotics have been taken in the 3 months prior the beginning of the intervention. The study was approved by the institutional review board of the A. O. Ospedali Papa Giovanni XXIII (Bergamo, Italy), and all enrolled subjects or tutors gave written informed consent in accordance with the sampling protocol approved.

We performed a double-blind trial by assigning, randomly, patients to groups given the probiotic preparation (n=9) or placebo (n=9) for 3 months. The probiotic group received an oral therapy with 1 sachets per day VSL#3 containing $4.5x10^{11}$ colony forming units of bacteria per sachet (VSL#3; Ferring, Milan, Italy). VSL#3 contains eight different strains of bacteria: *Streptococcus salivarius* subspecies *thermophilus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus delbrueckii* subspecies *bulgaricus* (24). There were 4 drop-outs, one in the probiotic group and three in the placebo group. An overview of the trial is illustrated in Figure 1.

3.2.2 Baseline and follow-up protocol

Baseline examination of the patients comprised a physical examination, peripheral blood measures and abdominal ultrasonography as well neuropsychiatric tests. After the 3 months, patients were subjected to a physical examination, and new neuropsychiatric tests. Faecal samples from enrolled subjects were collected, aliquoted and stored at −80°C until analysis.

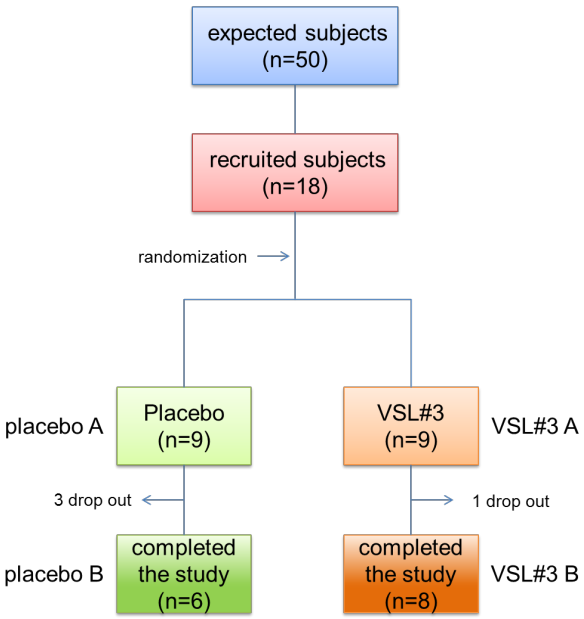

Figure 1. Flow chart showing the overview of the study

3.2.3 DNA extraction, PCR amplification of the V3-V5 region of bacterial 16S rDNA

Total DNA extraction from faecal samples (250 mg, wet weight) was performed using the FastDNA™ SPIN Kit for Feces (MP Biomedicals, Santa Ana, CA, USA) following manufacturer's instructions. DNA integrity and quality were checked on 1 % agarose gel TAE 1X and quantified with Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific). Samples were subjected to PCR amplification, Using the specific bacterial primer set 341F (5' CCTACGGGNGGCWGCAG 3') (43) and 806R (5' GACTACNVGGGTWTCTAATCC 3') (44) with overhang Illumina adapters targeting a ~460 bp fragment of the 16S rRNA variable region V3-V4. PCR amplification of each sample, was carried out using 25 µl reactions with 0.2 µM of each primer. In particular 12.5 µl of 2x KAPA HiFi HotStart ReadyMix, 5 µl forward primer, 5 µl reverse primer, were used in combination with 2.5 µl of template DNA (5 ng/ul). All PCR amplification was carried out, using a GeneAmp PCR System 9700 (Thermo Fisher Scientific) and the following steps: melting step – 95°C for 3 minutes (one cycle); annealing step – 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds (25 cycles); extension step – 72 °C for 5 minutes (1 cycle). The PCR products were checked on 1.5 % agarose gel and cleaned from free primers and primer dimer, using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA), following the manufacturer's instructions. Subsequently, dual indices and Illumina sequencing adapters Nextera XT Index Primer (Illumina), were attached by 7 cycles PCR (16S Metagenomic Sequencing Library Preparation, Illumina). The final libraries, after purification by the Agencourt AMPure XP system (Beckman), were analyzed on a Typestation 2200 platform (Agilent Technologies, Santa Clara, CA, USA) and quantified using the Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific). Finally, all the libraries were pooled in an equimolar way, in a final amplicon library and analyzed on the Typestation 2200 platform. Barcoded libraries were sequenced on an llumina® MiSeq (PE300) platform (MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18).

3.2.4 Metagenomic data analysis

The sequences were assigned to samples, according to sample-specific barcodes. This allowed collecting the FASTQ formatted files. Raw data will be submitted to European Nucleotide Archive before publication. Reads were processed using the QIIME pipeline (45), where open reference operational taxonomic unit (OTU) picking was performed in using usearch61 (46). Alpha (within-sample richness) and beta-diversity (between-sample dissimilarity) estimates were computed using the phyloseq R package (47). Permutational MANOVA (PERMANOVA) was performed on the UniFrac distances and Bray-Curtis dissimilarity using the adonis function of the vegan R package with 999 permutations, and p-values were corrected using the Bonferroni correction. The non-parametric Wilcoxon rank-sum test was used for the comparison of relative abundances of microbial taxa between groups, and the resulting p-values were corrected for multiple testing controlling the false discovery rate (48) at all taxonomic levels taken into account.

Starting from a table of OTUs, we obtained the final output from metagenome prediction as an annotated table of predicted gene family counts for each sample. All statistical analyses were performed using R (R: A language and environment for statistical computing, https://www.r-project.org/).

3.3 Results

3.3.1 Characteristic of study population

Eighteen patients fulfilling inclusion criteria were recruited for the study between March 2013 and April 2016. All patients underwent the baseline evaluation and were randomly assigned to the VSL#3 (1 sachet /daily) or placebo groups. Four patients discontinued the treatment for personal reasons. Therefore the final study population comprised 14 patients (VSL#3, n=8; placebo, n=6) (Figure 1). The characteristics of these patients at baseline are shown in Table 1. The data reported are only for the intent-to-treat population.

Psycometric tests, including visual motor intergration (VMI) test, to evaluate memory, neuromotor function and attention were measured at baseline and after 3 months of VSL#3 or placebo.

Blood ammonia levels prior and post treatment are shown in Table 2. We found that there were no significant differences between the treated and placebo groups. Similarly, no significant changes were measured in the other blood parameters measured (Table 3).

Table 1. Baseline characteristics of the final study population

	VSL#3 group	Placebo group	All patients (n=14)
Age [years; median (range)]	10 (6-14)	10 (4-18)	10 (4-18)
Aetiology [n(%)]			
Cavernome portal hypertension	1 (12.40%)	1 (16.66%)	2 (14.28%)
Portal thrombosis		3 (50%)	3 (21.42%)
Congenital hepatic fibrosis		1 (16.66%)	1 (7.14%)
Biliary atresia	6 (75.00%)	1 (16.66%)	7 (49.98%)
Sclerosis cholangitis	1 (12.50%)		1 (7.14%)
Varices [n(%)]			
No	5 (62.50%)	5 (35.70%)	8 (57.12%)
Small	1 (12.50%)	2 (14.28%)	3 (21.42%)
Large	2 (25.00%)	1 (7.14%)	3 (21.42%)
Haemoglobin level [g/dl, median (range)]	13.0 (12.5-14.3)	11.7 (10.3-13.3)	12.75 (10.3-14.3)
White cell count, per mm³ [median (range)]	3.24 (2.22-3.75)	3.58 (1.51-8.02)	3.25 (2.24-8.02)
Platelet count, per mm³ [median (range)]	92.1 (44-131)	69 (40-198)	71 (26-198)
Serum bilirubin level [g/dl, median (range)]	1.11 (0.4-2.8)	0.74 (0.4-1.2)	0.95 (0.4-2.8)
International normalized ratio [median (range)]	1.18 (0.93-1.29)	1.28 (1.1-1.42)	1.24 (0.93-1.4)
Serum abumin level [median (range)]	4198 (3740-4480)	4489 (4428-4676)	4293 (3489-4676)
Alanine aminotransferase ALT [IU/l, median (range)]	56.9 (18-185)	24.5 (22-144)	53.5 (20-185)
Aspartate aminotransferase AST [IU/l, median (range)]	51.5 (22-143)	30.5 (19-210)	48.5 (19-210)
Gamma-glutamyl transferase GGT [IU/l, median (range)]	152.4 (23-502)	22.5 (6-330)	53.5 (6-502)
Alkaline phosphatase ALP [IU/l, median (range)]	359.7 (227-592)	171 (46-799)	234 (46-799)

Table 2. Blood ammonia levels (μmol/l) before (A) and after (B) VSL#3/placebo treatment

	Median value	range	Mean ± SD
Placebo A	26	15-119	37 ± 36.7
Placebo B	28	11-108	38 ± 36.7
VSL#3 A	32	18-96	38.6 ± 23.8
VSL#3 B	34	19-76	39.5 ± 18.4

Table 3. Blood parameters at the end of the study

	VSL#3 group	Placebo group
Haemoglobin level [g/dl, median (range)]	12.3 (11.7-13.9)	11.8 (9.7-13.5)
White cell count, per mm^3 [median (range)]	2.76 (1.62-4.07)	4.26 (1.27-9.32)
Platelet count, per mm^3 [median (range)]	84 (36-139)	72 (60-246)
Serum bilirubin level [g/dl, median (range)]	0.9 (0.4-2.4)	0.7 (0.4-1.4)
International normalized ratio [median (range)]	1.22 (0.91-1.36)	1.38 (1.0-1.48)
Serum abumin level [median (range)]	4233 (3961-4425)	4420 (3677-4778)
Alanine aminotransferase ALT [IU/l, median (range)]	55 (23-232)	23.5 (17-192)
Aspartate aminotransferase AST [IU/l, median (range)]	66 (27-149)	33.5 (17-205)
Gamma-glutamyl transferase GGT [IU/l, median (range)]	55 (18-197)	20.5 (5-300)
Alkaline phosphatase ALP [IU/l, median (range)]	271 (46-560)	177 (85-907)

3.3.2 Determination of the effects of probiotic VLS#3 supplementation on gut microbiota

Faecal samples were collected at the beginning of the experiment before intervention and after the treatment. The bacterial gut microbiota was profiled using Illumina high-throughput sequencing of the V3-V4 region of the 16S rRNA gene. Bacterial richness within each sample (α-diversity) of the 4 groups, VSL#3 and placebo groups, both before (A) and after the treatment (B) was calculated. Three different alpha-diversity estimators were used, namely the observed number of OTUs, the Chao1 index and the Shannon entropy index. The bacterial gut microbiota of subjects did not change significantly upon treatment with either VSL#3 or placebo (Figure 2 and Table A1, Appendix B).

To identify possible differences between the bacterial components of the gut microbiota of the VSL#3 treated patients compared to the placebo, we calculated the beta-diversity of the samples using the unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity. The Principal Coordinates Analysis (PCoA) based on these measures (Figure 3 and Figure S1) revealed that the gut microbiota of probiotic treated patients was not distinct from those of the control group (Table 4).

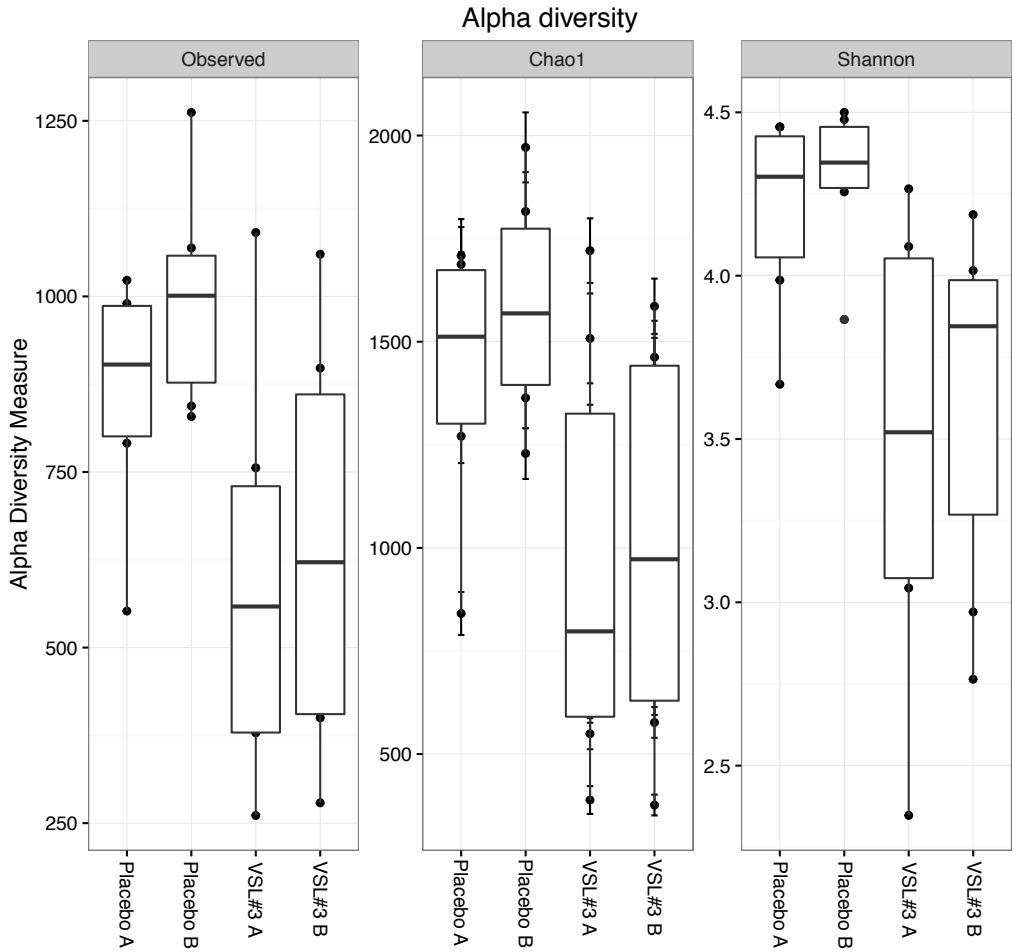

Figure 2. Measure of bacterial diversity. Alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index. No statistically significant differences have been found. The body of the box plot represents the first and third quartiles of the distribution, and the median. The whiskers extend from the quartiles to the last datapoint within 1.5× IQR, with outliers beyond represented as dots. Placebo A, placebo at baseline; placebo B, placebo at 3 months; VSL#3 A, VSL#3 at baseline; VSL#3 B, VSL#3 at 3 months.

However, the analysis of β-diversity among VSL#3 treated patients and placebo groups after the 3 month-therapy revealed significant differences (VSL#3 B vs placebo B, p=0.036, PERMANOVA, Figure 2 and Table 4). Surprisingly no significant difference was detected when comparing the gut microbiota of patients before and after VSL#3 treatment (Figure S1 and Table 4). To identify the taxa that were differentially represented in the VSL#3 group and placebo subjects, we compared the relative abundances between these two groups at different taxonomic levels (Tables A2-A5, Appendix B).

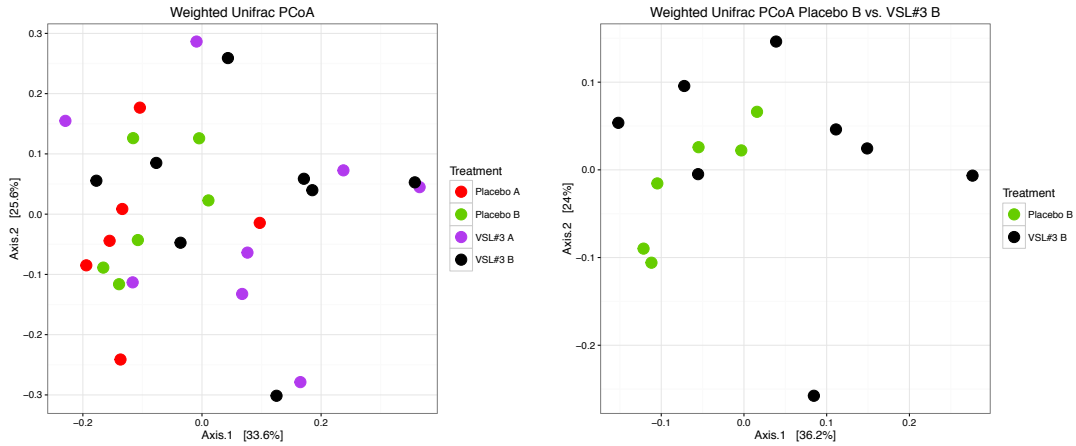

Figure 3. PCoA plots of bacterial beta-diversity based on the Weighted UniFrac distance according to groups of treatments (*left*) and groups of treatments after the 3 month-therapy (*right*). Placebo A, placebo at baseline; placebo B, placebo at 3 months; VSL#3 A, VSL#3 at baseline; VSL#3 B, VSL#3 at 3 months.

Table 4. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the
unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity according to individuals' treatments (VSL#3 or placebo)

	Metric	***F***	R^2	***p* value***
Treatments	Unweighted UniFrac	1055	0.11651	0.405
	Weighted UniFrac	1.247	0.13485	0.087
	Bray-Curtis	10.133	0.11243	0.43
Placebo A vs. Placebo B**	Unweighted UniFrac	0.32351	0.03134	0.917
	Weighted UniFrac	0.58886	0.05561	0.964
	Bray-Curtis	0.62049	0.05842	0.864
VSL#3 A vs. VSL3 B	Unweighted UniFrac	0.14861	0.0105	0.969
	Weighted UniFrac	0.40156	0.02788	0.998
	Bray-Curtis	0.20371	0.01434	0.997
VSL#3 B vs Placebo B	Unweighted UniFrac	1.7823	0.12932	0.081
	Weighted UniFrac	1.6989	0.12402	*0.036*
	Bray-Curtis	1.408	0.10501	0.075

*Bonferroni corrected p-values
**Placebo A, placebo at baseline; placebo B, placebo at 3 months; VSL#3 A, VSL#3 at baseline; VSL#3 B, VSL#3 at 3 months.

The analysis did not show any statistically significant increase or decrease in the relative abundance of any phylum, genera or OTU in the VSL#3 treated group compared to placebo or between the groups before treatment. However, there was a trend towards an increase in Actinobacteria in the VSL#3 group after the 3 months treatment (VSL#3 B)

compared to the baseline (VSL#3 A) (Figure 4). In the same group, a trend towards decreased Bacteroides relative abundance was observed, as well as a slight increase of *Ruminococcus*, *Faecalibacterium* and *Streptococcus* (Figure 4).

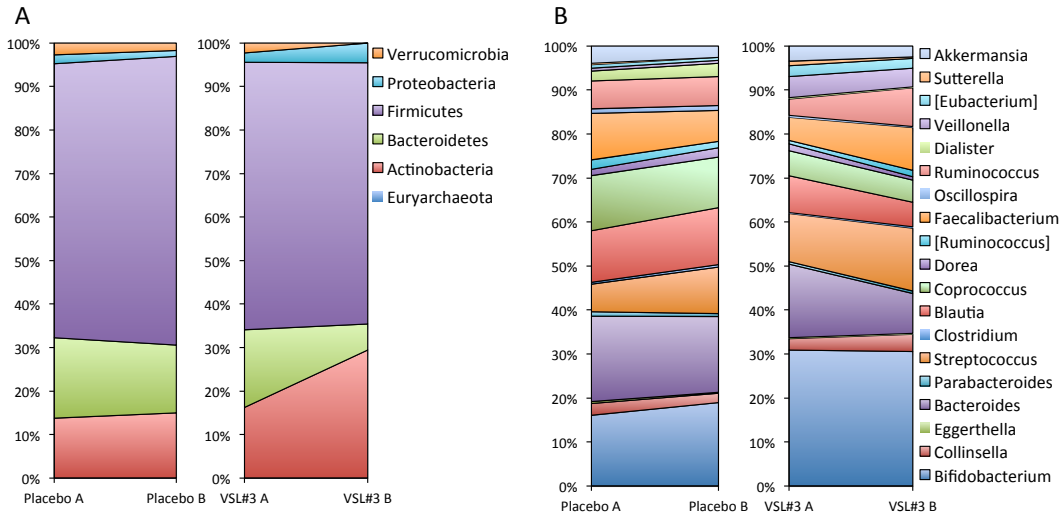

Figure 4. Relative abundance of microbial taxa at phylum (A) and genus (B) level. Only taxa with abundance > 0.001 are depicted. Placebo A, placebo at baseline; placebo B, placebo at 3 months; VSL#3 A, VSL#3 at baseline; VSL#3 B, VSL#3 at 3 months.

To investigate better the absence of any significant changes in terms of bacterial abundance after the VSL#3 treatment, we calculated the β-diversity of the subjects prior oral supplementation of VSL#3 or placebo. The unweighted UniFrac distance revealed that the two groups were different at the baseline (Figure 5A and Table 4). We then calculated the β-diversity of the subjects according to the different aetiologies (Figure 5B and Figure S2). The indices revealed that the patients distributed accordingly to their aetiology.

3.4 Discussion

Many liver and vascular diseases cause portal vein hypertension in children. Portal vein hypertension may give rise to severe and life-threatening complications, including haemorrhaging from oesophageal varices, ascites, hepatopulmonary syndrome, portopulmonary hypertension and HE. The effective prevention and management of portal hypertension and its complications, therefore, are important goals for improving health related outcomes and quality of life in affected children.

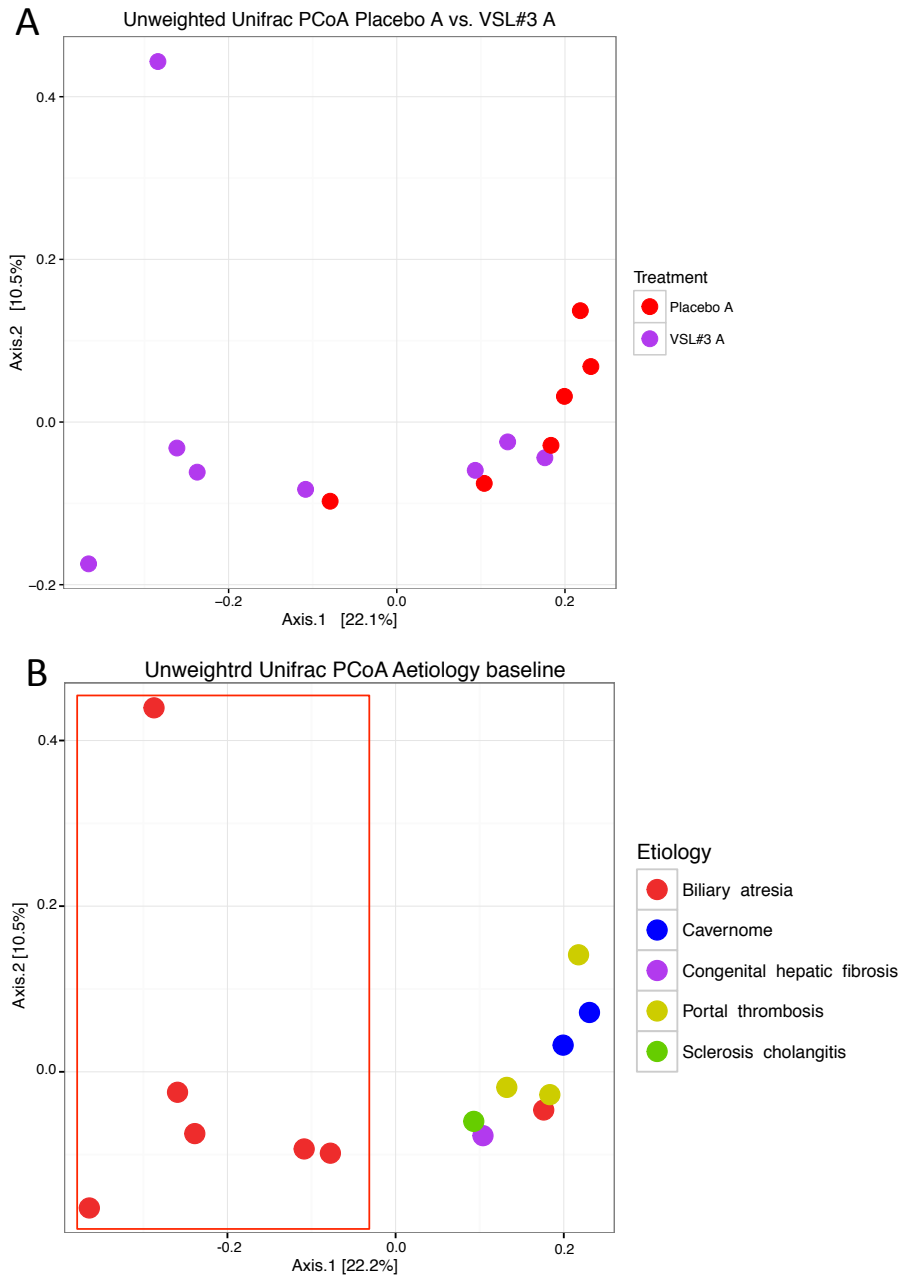

Figure 5. PCoA plots of bacterial beta-diversity. (A) Unweighted UniFrac distance calculated according to groups of treatments at the baseline. Placebo A, placebo at baseline; VSL#3 A, VSL#3 at baseline. (B) Unweighted UniFrac distance calculated according to the aetiologies of the population.

Table 4. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the

unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity. Placebo A, placebo at baseline; VSL#3 A, VSL#3 at baseline

	Metric	*F*	R^2	*p* value*
VSL3 A vs Placebo A**	Unweighted UniFrac	1.3029	0.09794	0.281
	Weighted UniFrac	1.5699	0.11569	*0.04*
	Bray-Curtis	1.2323	0.09313	0.158

*Bonferroni corrected p-values
**Placebo A, placebo at baseline; VSL#3 A, VSL#3 at baseline

As portal hypertension worsens, it is associated with a reduced ability of the liver to detoxify compounds such as ammonia absorbed from the gut. This in turn can lead to the onset of Minimal HE (MHE) (49,50), the mildest form of the spectrum of HE, the neurocognitive impairment in cirrhosis and/or portosystemic shunting (51). MHE impairs daily functioning, attention, speed of information processing, motor abilities, work capability, coordination and learning ability (51,52). The prevalence of MHE in adults with chronic liver disease ranges from 30-84% (53). It also predisposes to the development of overt HE, increased falls and increased mortality. This results in impaired quality of life and its early detection and treatment are mandatory. Two studies in 30 and 67 chronic liver disease children found MHE in 57% and 50.6 % cases, respectively (38,39). Improvement of cognitive function remains the main goal of the MHE treatment. Most of the available MHE therapies concentrate on reducing the serum ammonia levels by decreasing its production in the intestine and increasing its elimination. Prebiotics (34,54–56), antibiotics (16,57,58), L-ornithine L-aspartate (59), branched aminoacids (60), probiotics and synbiotics (23) as well as a low protein diet have all been shown to improve psychometric performance and quality of life.

The efficacy of probiotics in the treatment of liver disease and HE has been largely investigated in adults (10). In several human intervention studies in adults affected by HE provided probiotics have been linked to the amelioration of MHE symptoms (21–23,29,30) and the prevention of manifesting OHE (33,34). Despite the suspicion that probiotic effects on disease may be related to modulation of gut metabolism and consequent reduction in blood ammonia levels and endotoxemia (9–11), a clear association between modulation of the gut microbiota and amelioration of clinical conditions is still missing. Contrasting results directly linking the dysbiosis and the improvement of cognitive function in HE therapies have also been observed in response to other clinical therapies such as lactulose or rifaximin (61–65). This suggest the potential activity of these therapeutically approaches through changes gut bacterial function or metabolism, rather than microbiota composition.

Here, we studied the gut microbiota modulating effect of a 3-month oral administration of the probiotic mixture VSL#3 in paediatric and young adult subjects affected by portal hypertension manifesting with MHE. VSL#3 treatment did not promote a decrease in blood ammonia levels nor a significant change in the relative abundances of specific bacterial taxa. Our pilot intervention study suffer from the small number of

subjects recruited (14 out of 50 expected) and potential of the different underlying pathological causes of portal vein hypertension to influence baseline variation in gut microbiota composition. In fact, we did observe distinct microbiota profiles for the different underlying pathologies. Indeed, 7 out of 14 subjects were affected by biliary atresia and 6 out of those 7 were randomly included in the VSL#3 treatment groups. This aspect might therefore increase variation greatly and lower our ability to effectively measure the microbiota modulation of VSL#3. Liver disease results in qualitative (dysbiosis) and quantitative (bacterial overgrowth) changes of the intestinal microbiota, as recently indicated also for primary sclerosing cholangitis (66), one of the underlying pathologies present in our study. Similarly, it has been shown that the gut microbiota differed with respect to the pathological aetiology of liver disease (67,68). Hepatitis-B-virus related cirrhosis and primary biliary cirrhosis bear a different duodenal mucosa microbiota, characterized by a different abundance of Neisseria and Gemella (69). We are now assessing if the different aetiologies leading to portal intervention could account by themselves for distinct and diverse bacterial communities between patients. However, the 3 month-supplementation of VLS#3 did show a trend towards the increase in Actinobacteria and a concomitant decrease in Bacteroidetes. The results suggested also a mild decrease in *Bacteroides* relative abundance, as well as a slight increase of *Ruminococcus* and *Faecalibacterium*. *Ruminococcus* has been previously associated to secondary bile acids production and decrease in severity of cirrhosis progression (70) while *Faecalibacterium* abundance has been shown to be increased by lactulose treatments (71,72). To better address the potential of using the probiotic VSL#3 in the treatment of portal hypertension and HE in children a larger study with a more potent stratification for different underlying liver disease is therefore needed. However, according to previous studies (36,73,74), the 50 patients we initially planned to enroll for the study will be sufficient to observe microbiota changes as well as changes in the plasma ammonia or transaminase levels.

Thus, the investigation should not only to be restricted to the relative abundance of particular species but should also evaluate any changes in the gut microbiota metabolism, i.e the production of Short Chain Fatty Acids (SCFAs), which have been shown to be important for colonic health and may act on neuronal physiology (75–77). Indeed, 16S rRNA profiling flanked by a metabolomic approach would allow a better understanding of the link between microbiota modulation and disease symptoms.

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Supplementary Figures

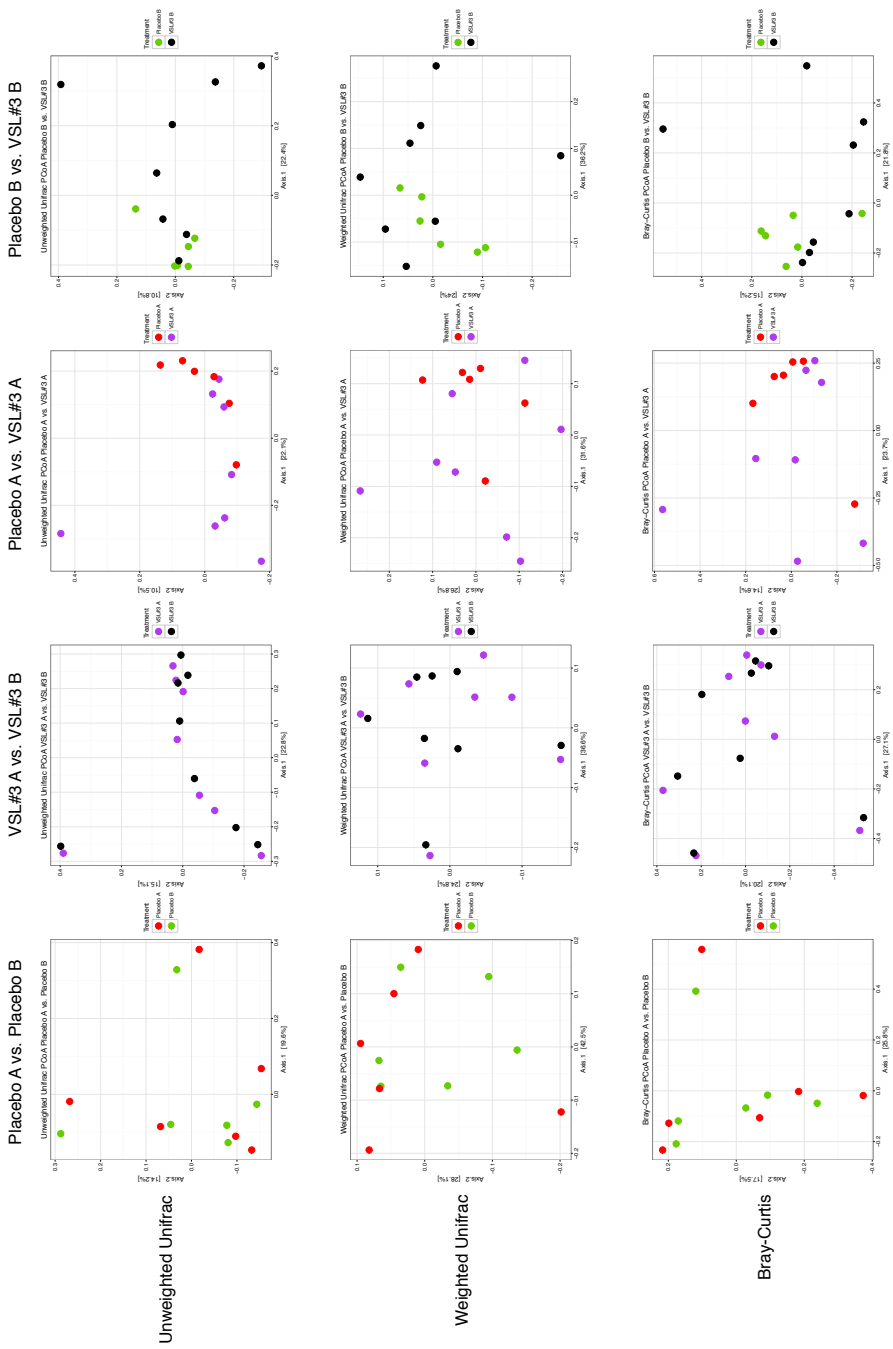

Figure S1. Measures of bacterial diversity. PCoA plots of bacterial beta-diversity based on Unweighted and Weighted UniFrac distances and the Bray-Curtis dissimilarity analyzed according to treatments. “A” baseline, “B” after 3 months.

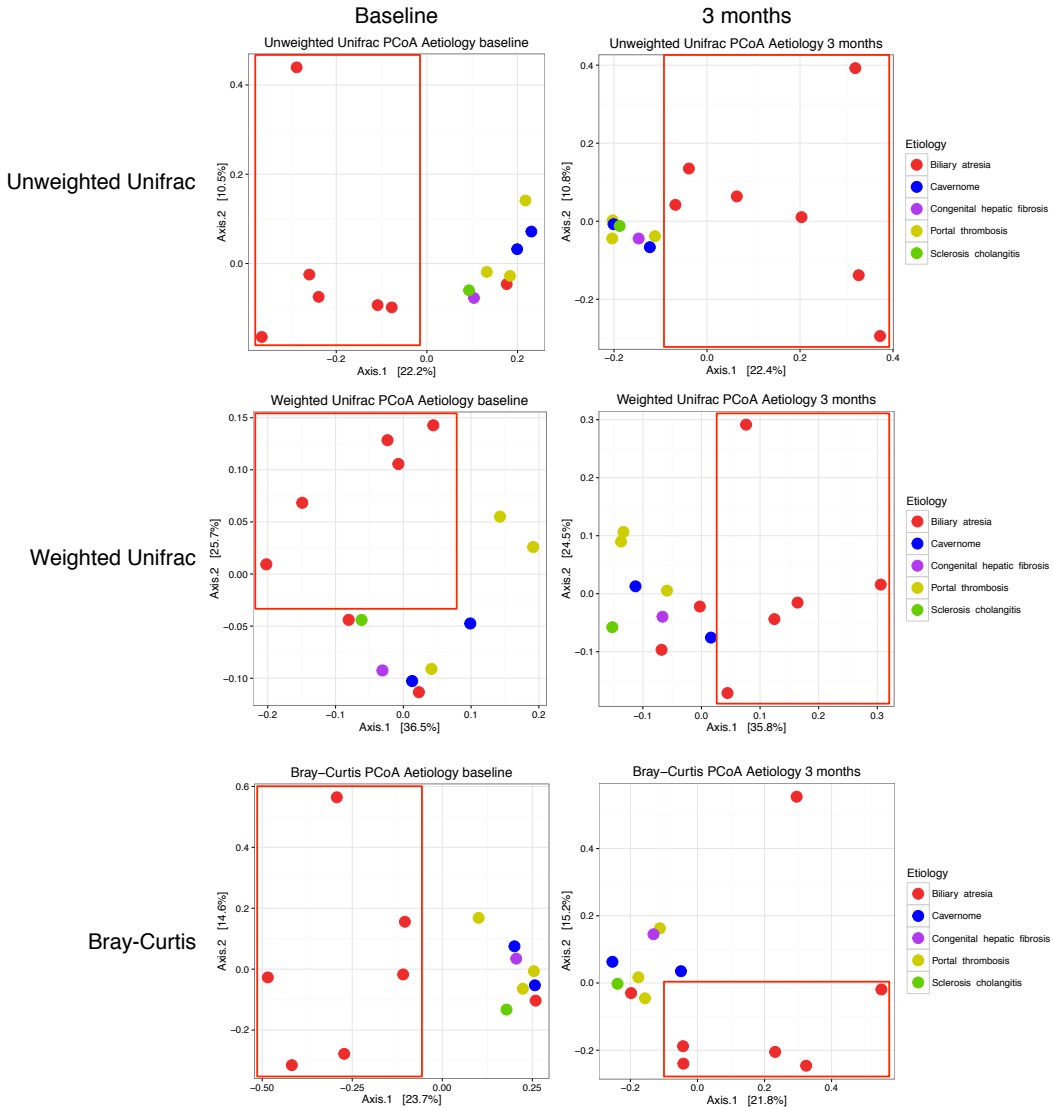

Figure S2. Measures of bacterial diversity. PCoA plots of bacterial beta-diversity based on Unweighted and Weighted UniFrac distances and the Bray-Curtis dissimilarity analyzed according to aetiologies.

Chapter 4

Probiotic characterization of high GABA producing strain *Lactobacillus brevis* FEM 1874

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Authors' contributions:
KMT conceived the idea
AM performed GABA level determination, DNA extraction and GAD operon characterization, RNA extraction and gene expression, pH and ox-bile resistant tests, measuring bacterial growth rate on different carbon sources, analysed the data and wrote the manuscript
EF, IC isolated the strain, performed the antibiotic resistance test and pancreatic fluids resistance test
KMT revised the manuscript

-Paper ready for submission-

Key words
probiotic, *Lactobacillus brevis*, oxbile, γ-aminobutyric acid, GABA, GAD genes

Abstract
γ-Aminobutyric acid (GABA) has strong potential for the food and pharmaceutical industries as a bioactive compound, with increasing evidence of its effects on the gut-brain axis. Different lactic acid bacteria are capable of producing GABA particularly strains of *Lactobacillus brevis*. In this study we characterized a *Lb. brevis* isolated from traditional alpine cheese for its ability to accumulate high levels of GABA in the culture medium and for other phenotypic traits important for probiotics. *Lb. brevis* FEM 1874 converted monosodium glutamate to GABA more efficiently compared to the type strain *Lb. brevis* DSM 20054, resulting in high amount of GABA. This ability seemed to be related to the higher transcriptional activation of the gene encoding for the glutamate (gad) decarboxylase antiporter (*gadC*) and regulator (*gadR*). *Lb. brevis* FEM 1874 performed well *in vitro* under the stress conditions mimicking the gastro-intestinal tract passage, being resistant to acid pH (pH 2.5) and growing on pancreatic fluid and 0.3% ox-bile. Compared to the type strain FEM 1874 expressed more efficiently the glutamate decarboxylase operon and was also able to produce high amount of GABA compared to the type strain These preliminary studies indicate that this strain holds promise as a starter for GABA-rich dairy fermented foods as well as a promising probiotic microorganism with potential to modulate the gut(microbiota):brain axis, portal vein hypertension and systemic inflammation through GABA production.

4.1 Introduction

γ-Aminobutyric acid (GABA) is a natural non-proteinogenic amino acid widely found in animals, plants and microorganisms (1–4). It represents the major inhibitory neurotransmitter of the vertebrate central nervous system (5), where it modulates the general excitability of neurons (6,7). GABA is involved in the regulation of cardiovascular conditions such as blood pressure and heart rate and in the sensation of pain and anxiety (8), moreover it controls different activity such as growth hormone secretion (9), protective effect against glycerol induced acute renal failure in rats (10) and anti-proliferative activity on colon carcinoma cells (11). GABA possesses several physiological functions such as improving brain function, antianxiety effects, tranquilizer effects, boosting fertility, diuretic effects, anti-diabetic effects and treatment of epilepsy (7,12). Alteration of the GABA system can lead to anxiety and depression (13,14). Aside from central nervous system, GABA is present also in many organs such as the pancreas, pituitary, testes, gastrointestinal tract, ovaries, placenta, uterus and adrenal medulla (15). Also immune cells may also produce GABA expressing GABA-A ion channels, GABA transporters and the GABA-B receptor (16). Indeed GABA is able to activate GABA-A ion channel in T cells and macrophages (17–19) and its application resulted in decreased cytokine secretion and

T cells proliferation (18–20). Moreover recent evidence support the potential of GABA derived from the gut to act as neuroactive molecule in the context of the gut-brain axis - the complex communication system established between the gut microbiota and the (central and peripheral) nervous systems (21,22).

Indeed, GABA is currently being investigated as a bioactive compound by both the food and pharmaceutical industries (1,4,23–25) and a several number of placebo controlled studies reported have been carried out to study the effects of an oral administration of GABA (26–31). A strong effort has been made in the formulation of GABA enriched foods especially fermented foods, including dairy products, soybean, kimchi and juice products, which could be used as potential GABA-delivery vehicles (32–37). Cheese is one great source of GABA, where GABA is produced during cheese ripening (38–40). One approach may therefore be to increase GABA levels in humans by consuming GABA-enriched food products. Isolation of GABA producing strains from diverse fermented food and from the human gut is providing considerable natural biotechnological solutions for the design of new GABA-rich fermented foods and for the selection of next generation, efficacious, probiotic strains (25,40–42).

As defined by the Food and Agriculture Organization and the World Health Organization, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Other definitions advanced through the years have been restrictive by specification of mechanisms, site of action, delivery format (e.g. food) or host (43). To accumulate in the intestinal tract, probiotics must first survive the various conditions specific to the digestive system, such as low pH in the stomach and the presence of bile acids in the intestines: these represent the most important properties of a probiotic (44). Particularly bile secreted into the duodenal section of the small intestine is known to reduce the viability of probiotics, and the ability to tolerate bile is one of the requisite characteristics of successful probiotics (45,46). Probiotics have been shown to exert a wide range of effects, such as enhancing immune function (43,47–50), improving metabolic disorders (51–54) and being effective against pathogens, such as *Clostridium difficile* (55). Probiotic efficacious have been shown to be species and even strain dependent since different bacterial strain can affect host via diverse modes of action (56–58). Of consequence, probiotics targeted toward unique outcomes and functionalities are now be demanded as next generation probiotics (NGP) and put through rigorous and properly designed human intervention studies. A large number of potential novel probiotic candidates are being isolated from the dominant members of the adult microbiota as well as from traditional fermented food, as potential next-generation probiotics (59).

Probiotic intervention targeting the microbiota-gut-brain axis to modulate behavior has recently been reported. The probiotic *Bifidobacterium longum* (Bl NCC3001) has been shown to normalize behavior and CNS biochemistry (60,61) in mice with mild colitis, an effect also mediated though the vagus nerve (61,62). By contrast, *Lactobacillus rhamnosus* NCC4007 (LGG) was not able to improve anxiety-like behavior (60). However, another strain of *Lb. rhamnosus*, namely Lr JB-1, was able to induce a direct effect on behavioural

and physiological responses in a vagus nerve-dependent manner (21). *Lb. rhamnosus* JB-1 promoted an anxiolytic–antidepressant-like effect through alterations in the expression of GABA receptors, such as GABAAα2, GABAAα1, and GABAB1b (21). Indeed, GABA receptors targeting represents a goal for improving brain function. Identification of bacteria able to produce high GABA levels and bearing features of a probiotic is a viable approach for designing efficacious next generation probiotics targeting the gut:brain axis.

GABA is the end product of the α-decarboxylation of glutamic acid by lactic acid bacteria (LAB) and several GABA-producing LAB species isolated from traditional fermented food and beverages have been reported. These include *Lactobacillus paracasei* (25) (40,63), *Lb. buchneri* (6,37,64), *Lactococcus lactis* (39,40), *Lb. delbrueckii* subsp. *bulgaricus* (40), *Lb. plantarum* (40) and *Lb. brevis* (37,40,42,65–67). Cheese represents a rich source of LAB with potential GABA producing properties (38–40). The results of these findings offer potential alternatives to take advantage of GABA's health benefits through GABA-enriched cheeses. In this work, we characterized an isolate from "Nostrano-cheese", typical of the Trentino province (north, alpine area) in Italy, *Lb. brevis* FEM 1874, for its ability to produce GABA and for some characteristics considered important for a probiotic strain (68,69). The high GABA producing *Lb. brevis* FEM 1874 strain possesses a Generally Regarded As Safe (GRAS) status – as a *Lactobacillus* - and is able to survive gastrointestinal (GI) tract conditions, which makes it a good candidate as a starter ingredient for functional food and potential of next generation probiotic with specific mode of action based around its GABA producing capability and modulation of the gut:brain axis.

4.2 Material and methods

4.2.1 Reagents

All media constituents were purchased from Oxoid Ltd. (Basingstoke, UK) and Sigma Aldrich (Milan, Italy), chemicals were purchased from Sigma Aldrich (Milan, Italy). VSL#3 (sachets) were kindly provided by Ferring (Milan, Italy).

4.2.2 Bacterial strains, culture medium and growth conditions

The stock culture collection of *Lb. brevis* FEM 1874 and DSM 20054 were maintained at -80°C in 20% v/v glycerol. Bacteria cells were propagated twice in MRS broth (Oxoid Ltd., Basingstoke, UK) by incubation at 37°C for 16 h before each experiments. VSL#3 powder was washed three times with PBS, counted and used the appropriate concentration for the different assays.

4.2.3 γ-Aminobutyric Acid (GABA) production and quantification

Glutamate decarboxylase (GAD) activity of *Lb. brevis* FEM 1874 and the production of GABA were measured as reported by Nomura et al. (70). Briefly, *Lb. brevis* FEM 1874 and DSM 20054 were grown in MRS for 24 h at 37°C temperature, VSL#3 was

washed three times with sterile 1X PBS (pH 7.4). Cell cultures were then centrifuged (8600 rcf for 15 min at 4°C), washed twice with sterile PBS, and suspended in sterile 0.85% w/v NaCl solution in order to achieve the A620 nm value of 2.5. 100 µL of cell suspension was then mixed with 900 µL of 50mM sodium acetate buffer (pH 4.7) containing 7.0 mM L-glutamate and 0.1 mM pyridoxal phosphate. The reaction mixture was incubated for 24 h at 37°C and filtered through a 0.22 µm pore size filter (Minisart, Sartorius Stedim Biotech, Goettingen, Germany). The sample, diluted 10 times with sodium tetraborate 0.1 M (pH adjusted to 10.5) and added to glycine, as internal standard to a final concentration of 10 mg/L, was stored at −20°C before the analysis. L-Glutamic acid, glycine, and GABA were quantified as o-phthalaldehyde (OPA) adducts. The detection limit for GABA was estimated at 0.025 mg/L (3 times the standard deviation of the GABA contents measured repeating 10 times the analysis of a sample at unquantifiable content).

4.2.4 GAD genes sequencing

From *Lb. brevis* FEM 1874 overnight broth culture DNA was extracted with QIAamp DNA Blood Mini Kit (QIAGEN, Milan, Italy) following the manufacturer protocol. PCR amplification for the GABA genetic locus (*gadR*, *gadA*, *gadC* and *gadB*), were performed by using of specific primers (see Supplementary Table S1). PCR reactions were carried out in a 2720 Applied Biosystems Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Amplified products were subsequently purified using the Promega PCR and Gel Clean Up system kit according to the manufacturer's instructions (Promega Corporation, Milan, Italy). Sequencing was carried out by Sequencing Platform Unit, Fondazione Edmund Mach (San Michele a/A, Trento, Italy). The identifications were refined by BLAST (1 www.ncbi.nlm.nih.gov/BLAST) and Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/) alignment of the GAD DNA sequences to the reference genome. The sequence of *gadR*, *gadA*, *gadC* and *gadB* loci were deposited in GenBank database. Data submission is pending.

4.2.5 GAD genes expression

Lb. brevis FEM 1874 and DSM 20054 were inoculated in MRS in presence of 30 mg/ml of monosodium glutamate for 12 hours. Total RNA was extracted from cultures at 0, 3, 7 and 12 hours using TriZol® (LifeTechnologies, Monza, Italy), according to the manufacturer's instructions. The sample was reverse transcribed using SensiFAST cDNA Synthesis Kit (BioLine AUROGENE s.r.l., Rome, Italy) and cDNA products were amplified in the presence of the specific primers for *gadR*, *gadA*, *gadB* and *gadC* as well as for the housekeeping gene *tuf1* (see Supplementary Table S2). The conditions were chosen so that none of the RNAs analysed reached a plateau at the end of the amplification protocol, i.e. they were in the exponential phase of amplification. Each set of reactions always included a no-sample negative control. The PCR products were loaded onto Ethidium Bromide-stained, 1% agarose gels. A 1 Kbp DNA ladder molecular weight

marker (Life Technologies, Monza, Italy) was run on every gel to confirm expected molecular weight of the amplification product. After images acquisition, quantification of the bands was performed using ImageJ software (71). Band intensity was expressed as relative absorbance units. The ratio between the sample RNA to be determined and the housekeeping reference gene *tuf1* was calculated to normalize for initial variations in sample concentration and as a control for reaction efficiency. Mean and standard deviation of all experiments performed were calculated after normalization to *tuf1*. The experiment was performed in triplicate.

4.2.6 Tolerance to pH, oxbile and pancreatic fluid

Effect of low pH was studied by the method of Tsai et al. (72). Briefly, one millilitre of culture containing about 10^9 CFU/ml of LAB was transferred into 9 ml phosphate-buffered saline (PBS). The pH was adjusted to 2.0, 2.5 and 3.2 using 0.1 N HCl and cells incubated at 37°C for 3 h. Control was performed at pH 7.2. After incubation, serial dilution plating on MRS agar were performed to determine viable bacterial counts. Plates were incubated anaerobically at 37°C for 48 h and acid tolerance was estimated by comparing the viable LAB bacteria counts in MRS agar for surviving cells. Data are presented as log(CFU/ml) ± standard deviation. Five independent experiments were performed.

Tolerance for bile acids was performed on LAB cells exposed to low pH. After the 3 h treatment described above, cells were centrifuged (5000g, 5 min), washed with PBS (pH 7.2) and then grown in 9 ml MRS broth with and without 0.3% (w/v) Oxgall bile (Sigma Aldrich, Milan, Italy) for 3, 12 and 24 h. Bile tolerance was estimated by comparing the viable LAB bacteria count in MRS with and without bile salt. Data are presented as log(CFU/ml) ± standard deviation. Three independent experiments were performed.

Tolerance for pancreatic fluid was tested by inoculating actively growing bacteria (10% v/v inoculum size) to the test medium [150 mM $NaHCO_3$ and 1.9 mg/ml pancreatin (Sigma, USA); pH 8.0]. The cultures were kept for 3 h in a shaking water bath (Certomat WR, B. Braun, Melsungen, Germany) at 37°C. Survival of LAB strains was examined by plating on MRS agar after 0, and 3 h of incubation. Data are presented as log(CFU/ml) ± standard deviation. Three independent experiments were performed.

4.2.7 Growth on different carbon substrates

Lb. brevis FEM 1874 growth rate to different carbon substrates was monitored by supplementation of PY (0. 2% w/v Peptone, 0.05% w/v Bacto Yeast Extract, 3% w/v NaCl, 0.5% w/v $MgCl_2$ x $6H_2O$, 0.0005% w/v $CaCl_2$ x $2H_2O$, 0.0005% w/v Na_2MoO_4 x $7H_2O$, 0.0004% w/v $CuCl_2$ x $2H_2O$, 0.0006% w/v $FeCl_3$ x $6H_2O$) broth with 1% of glucose, fructose, lactate, lactulose, inulin and arabinogalactan. Optical density has been measured spectrophotometrically at 650 nm over 24 hours. Specific growth rate has been calculated

using the formula $N_t = N_0 e^{\mu t}$, where: N_t was the OD at 24 hours; N_0, the OD at time 0; μ, the specific growth rate and t, the time passed (24 h).

4.2.8 Antibiotic susceptibility test

Antibiotic resistance to ampicillin, vancomycin, gentamicin, erythromycin, clindamycin, and tetracycline was assessed using the strip test M.I.C.Evaluator (Oxoid Ltd., Basingstoke, UK) following the manufacturer's instruction. The strips consist of a gradient of stabilised antimicrobial covering 15 doubling dilutions. M.I.C.E. strips were used on a pre-inoculated agar plate, with formation of defined concentration gradient in the area around it. Minimum Inhibitory Concentration (MIC) was determined at the border of growth inhibition around the strip. Values were compared to the guidelines for facultative heterofermentative lactobacilli as indicated in the "Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance" by the European Food Safety Authority (EFSA) (73).

4.2.9 Statistics

Paired t-test was used to compare differences between the effect of a particular stress condition and the control, or between different gene level expression. The level of significance was set at $p<0.05$.

4.3 Results

4.3.1 *Lactobacillus brevis* FEM 1874 GABA production

GABA is produced primarily from the irreversible α-decarboxylation of L-glutamate by the enzyme glutamate decarboxylase (GAD) (Cotter and Hill, 2003). In the intracellular glutamate decarboxylase (GAD) system, glutamate is imported into cells by the GABA antiporter, decarboxylated by intracellular GAD to produce GABA and subsequently GABA is exported from the cells via the antiporter (74–76). In *Lc. lactis* the GAD gene (*gadA*) and the glutamate/GABA antiporter gene (*gadC*) are part of an operon, positively regulated by the gadR protein (encoded by *gadR*) which recognises glutamate and induces gene expression (75).

Our previous study identified several cheese isolates capable of producing GABA (38). Starting from this preliminary information, we focused on *Lb. brevis* FEM 1874. Firstly, the GABA production rate of *Lb. brevis* FEM 1874 was compared to that of the type strain *Lb. brevis* DSM 20054 and the well-known probiotic mixture VSL#3 (77). VSL#3 is currently used as second line of intervention in prophylaxis of diseases affecting the gut:brain axis, such as liver and inflammatory bowel diseases (78–81). As reported in Table 1, after incubation at 37°C for 24 h, FEM 1874 was able to produce considerable higher quantity of GABA compared to the other strains examined ($p<0.00001$).

Table 1. GABA level production

	GABA (mg/L)
FEM 1874	262.06 ± 15.42
DSM 20054	78.27 ± 18.61*
VSL#3	9.39 ± 0.21$

*p<0.00001, FEM 1874 vs DSM 20054,
$ p<0.00001, FEM 1874 vs VSL#3

Two different GAD encoding genes have been characterized in different *Lb. brevis* stains, namely *gadA* and *gadB* (39–41,63). *gadA* is located adjacent to and downstream of the glutamate/GABA antiporter gene (*gadC*), commonly referred as *gad*CA. They form an operon with the operon regulator *gadR*, being immediately upstream of *gad*CA. *gadB* is located separately from the other *gad* genes (74). By the use of fourteen sets of primers based on the nucleotidic sequence of the reference strain ATCC367, the FEM 1874 operon and *gadB* sequences were amplified confirming the presence of each genetic locus involved in the GABA production within FEM 1874 strain. The gene sequences shared high similarity with the ATCC367 strain, revealing the absence of any polymorphisms in the operon system or in the antiporter, which could account for this strain ability to produce the high amount of GABA. We thus asked whether this high ability was related to an increase in gene expression of such genes in the presence of glutamate. The semiquantitative expression analysis of *gad* genes showed a different expression profile in *Lb. brevis* FEM 1874 and DSM 20054 genes, both in presence or absence of the operon inducer glutamate (Figure 1). While *gadA* and *gadB* genes are repressed in absence of glutamate, the presence of glutamate induced their expression in both strains. *gadA* and *gadB* were up-regulated to a greater degree by FEM 1874 than in the type strain (*gadA*, $p<0.05$; *gadB*, $p<0.01$). Interestingly, at 7 h of growth, FEM 1874 strain induced an higher expression of *gadC* gene (relative expression = 2.17±0.11, $p<0.01$), encoding for the antiporter in response to glutamate and of *gadR*, the gene encoding for the GAD operon regulator (relative expression = 1.96±0.05, $p<0.01$), which was down regulated in DSM 20054 strain (Figure 1).

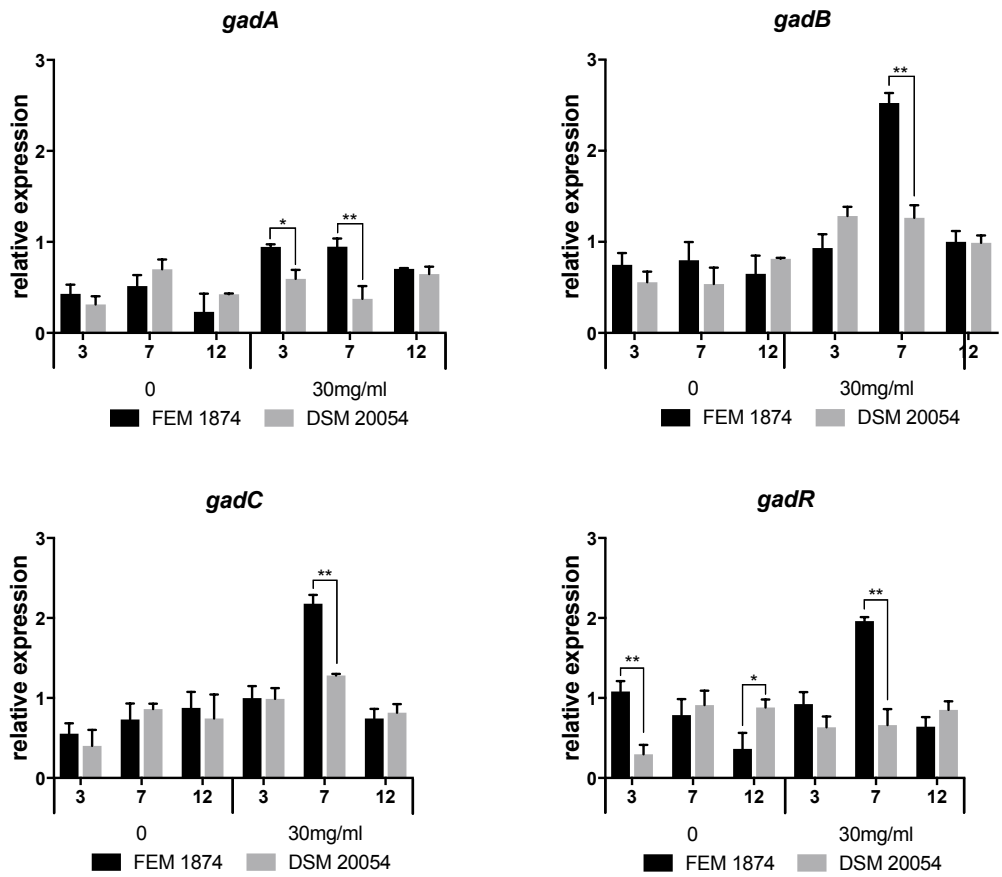

Figure 1. Expression levels of gad genes in response to 30mg/ml of monosodium glutamate. Semi-quantitative PCR has been used to assess the transcription level of the gad genes in FEM 1874 and DSM 20054 as described in Material and Methods section. Data are presented as fold increase with respect to the reference gene tuf1 (mean ± sd, N=3). *p<0.05, **p<0.01, paired t-test, FEM 1874 relative expression *vs* DSM 20054 relative expression.

4.3.2 *Lb. brevis* FEM 1874 resists to simulated gastrointestinal conditions

One of the characteristics required for being a probiotic is the ability to survive the gastrointestinal (GI) physiochemical environment. Therefore we measured the *Lb. brevis* FEM 1874 tolerance to acid pH, bile and pancreatic fluid, mimicking the acidic and liptolytic environment present along the GI tract. *L. brevis* FEM 1874 was more resistant to acid pH than the type strain DSM 20054, being able to survive to 3 hours exposure at pH 2.5 and 3.2, but not to pH 2.0 (Table 2).

We next explored the resistance of our isolate to bile acid (Table 3). FEM 1874 cells that survived pH 2.5 (3 h) acid treatment were cultured in MRS broth in the presence or absence of 0.3% Oxbile. *Lb. brevis* FEM 1874 cells were able to resist to bile salts, even with a fitness reduction over time, as shown by the cell number decline (Table 3).

Table 2. Analysis of acid tolerance (pH 2.0, 2.5 and 3.2) for *L. brevis* FEM 1874

strain	0 h	pH 7.2, 3 h	pH 3.2, 3 h	pH 2.5, 3h	pH 2, 3h
FEM 1874	9.64 ± 0.40[a]	9.09 ± 0.21	8.85 ± 0.64	7.75 ± 0.74	nd
DSM 20054	9.87 ± 0.70	8.61 ± 0.22	8.43 ± 0.37	n.d	n.d

[a]Bacterial counts are converted to log CFU/ml.

Table 3. Effect of bile salts on FEM 1874 after low pH treatment

Time (h)	0	3	12	24
MRS	5.38 ± 0.79[b]	4.16 ± 0.45	6.29 ± 0.51	8.55 ± 0.37
MRS + oxbile[a]	5.78 ± 1.13	2.03 ± 1.3*	2.3 ± 1.41**	3.12 ± 1.55***

[a]MRS + oxbile means MRS broth with 0.3% Oxgall.
[b]Bacteria counts are converted to log CFU/ml.;
*p < 0.05, **p < 0.01, ***p < 0.001, paired t-test, oxbile *vs* none.

FEM 1874 cells surviving the pH 2.5 acid treatment grew at rate comparable to the not pH-treated cells when transferred into MRS broth (Table 3). Moreover, incubation of FEM 1874 strain in growth medium containing pancreatic fluid had no effect on its viability and fitness (Table 4). The type strain DSM 20054 behaved in a similar manner and not statistically significant differences were observed between the survival of both strain in pancreatic fluid (Table 4).

Table 4. Resistance of FEM 1874 to pancreatic fluid

Time (h)	0	3
FEM 1874	7.84 ± 0.56[a]	7.92 ± 0.45
DSM 20054	8.11 ± 1.13	7.90 ± 0.51

[a] Bacterial counts are converted to log CFU/ml.

4.3.3 Growth rates on different carbon substrates

The specific grow rate of FEM 1874 was measured on commercial sugars and prebiotics, including inulin, lactulose and the dietary fibre arabinogalactan. Growth curves over time revealed that FEM 1874 is able to utilize all the carbon sources tested even though at different extents (Figure 2).

Growth rates between time 0 and 24 h have been calculated for each substrate as indicated in Material and Methods section (Table 5). The results indicate a preference of *Lb. brevis* FEM 1874 for glucose, fructose and arabinogalactan. No significant differences have been observed between growth rates in lactose and lactulose as well as among those calculated for glucose or fructose and arabinogalactan (Table 5).

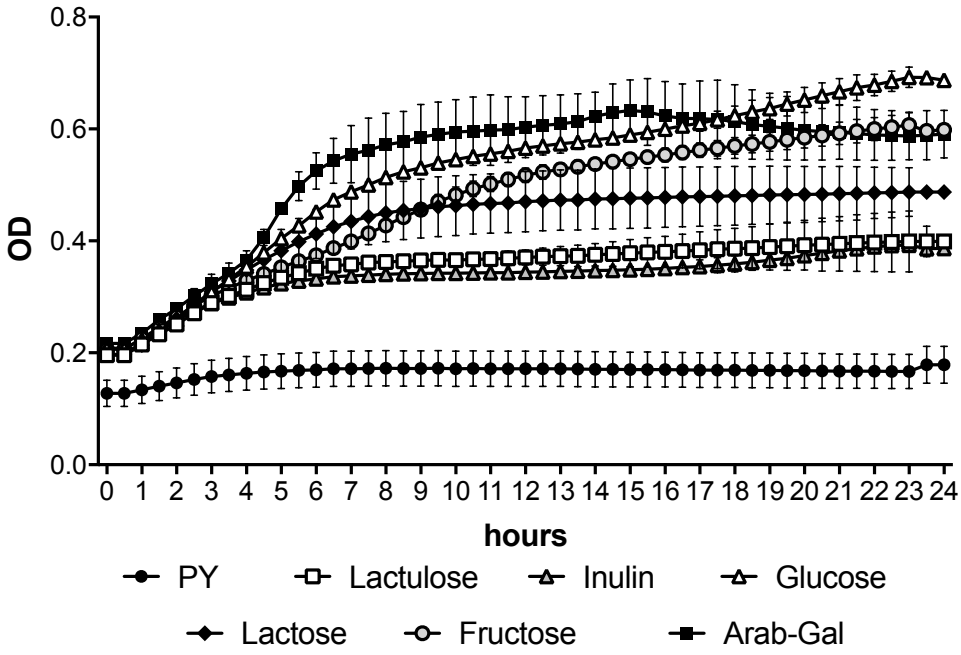

Figure 2. Fitness of FEM 1874 on different carbohydrates to be used as carbon sources. Growth curves on PY broth plus1 % w/v sugar have been determined over 24 h by means of optical density (OD) spectrophotometrically measures at 650 nm. Data are presented as mean ± sd (N=3). PY, Peptone Yeast; Arab-Gal, arabinogalactan

Table 5. Growth rates of FEM 1874 in different carbon substrates. *p-values, paired t-test, one sugar *vs* others; significant p-values are indicated in italics

	Specific growth rate, μ (h^{-1})	**Specific growth rate comparison**					
		lactulose *vs* others*	inulin *vs* others	glucose *vs* others	lactose *vs* others	fructose *vs* others	arab-gal *vs* others
lactulose	0.034 ± 0.0005		*0.023*	*0.004*	0.148	*0.012*	*0.004*
inulin	0.025 ± 0.0008	*0.023*		*0.0002*	*0.004*	*0.0002*	*0.001*
glucose	0.052 ± 0.0014	*0.004*	*0.0002*		*0.001*	*0.0008*	0.070
lactose	0.036 ± 0.0022	0.148	*0.004*	*0.001*		*0.003*	*0.009*
fructose	0.042 ± 0.0026	*0.012*	*0.0002*	*0.0008*	*0.003*		0.052
arab-gal	0.049 ± 0.001	*0.004*	*0.001*	0.070	*0.009*	0.052	

4.3.4 Antibiotic susceptibility testing

Regarding the safety assurance of probiotic organisms in food, FAO/ WHO guidelines (2002) suggest testing probiotic strains for antibiotic resistance. Primary testing of the antibiotic resistance patterns of *Lb. brevis* FEM 1874 strain was carried out by the E-strip method, following manufacturer's instruction. According to the sensitivity guidelines provided by EFSA (73), FEM 1874 was interpreted to be sensitive to ampicillin while

resistant to gentamicin, erythromycin, clindamycin, and vancomycin, with MICs being two, four, two and one times more the indicated cut off values (Table 6).

Table 6. Resistance of FEM 1874 to various antimicrobial agents

	CA	VA	DA	TE	AM	ER
cut off value (mg/ml)*	4	nr	1	8	4	1
MIC (mg/ml)	8	1	2	8	1	4

*Microbiological cut-off values as indicated by EFSA; CA, Gentamycin; VA, Vancomycin; DA, Clindamycin; TE, Tetracycline; AM, Ampicillin; ER, Erythromycin

4.4 Discussion

Beside the technological relevance of LAB in cheese production, there is a strong research interest to identify and characterize dairy strains with potential probiotic activities (82,83). The identification of strains with specific mode of action or biochemical trait capable of mediating specific host physiological responses represents the basis of a rational scientific selection of next generation probiotic strains designed to mediate specific health effects in the host. Targets will include microbiota-impacted physiological functions extending beyond the gut.

In the recent years, particular attention has been paid to high γ-aminobutyric acid (GABA) producing LAB strains (84) particularly from fermented food, including cheese (38,40) and fresh unpasteurized milk (85) or by strains isolated from the human intestinal tract (86). From a technological point of view, GABA in cheese may have a direct effect in the formation of holes thanks to the increased decarboxylase activity and consequent gas formation (87,88). This may sometimes be an unwanted phenotype depending on the type of cheese being produced. From a health perspective GABA has several well-characterized physiological functions in humans and other mammals. Moreover there is strong evidence that GABA derived from the gut can act as a neuroactive molecule in the context of the gut-brain axis (21,22).

In previous work, we isolated 276 strains from a specific raw cow milk "Nostrano-cheese", typical of the Trentino province (north, alpine area) in Italy. 71% bacterial strains were able to produce GABA (38). Upon investigation of GABA production, we found that one of these isolates, named *L. brevis* FEM 1874 possessed high glutamate decarboxylase (GAD) activity compared to the type strain DSM 20054 and the known probiotic mixture VSL#3 (89). As in *Listeria monocytogenes*, GAD activity in *Lb. brevis* may be critical for survival in acidic conditions and allows it to overcome the low pH stresses of fermented foods, gastric juice, volatile fatty acids in the GIT (90). The high GABA production from *Lb. brevis* has been confirmed by several individual studies on various *Lb. brevis* strains (91). GABA production represents therefore an important protection mechanism for these strains under acidic environments.

Two GAD-encoding genes, named *gadA* and *gadB* are present in *Lb*. *brevis* (74,91). The phylogenetic distance between *gadA* and the other GADs gene in *Lb*. *plantarum*, *Lc*. *lactis*, *Lb*. *reuteri* and *Lb*. *fermentum* suggests the occurrence of an independent evolution in *Lb. brevis* (91), and only *gadA* gene is present in the gad operon in *Lb*. *brevis*. *gadB* is located distantly from the *gad* operon. The high GABA production by *Lb. brevis* FEM 1874 appears not to be due to mutation in these genes as we identified the presence of the intact *gad* operon at the genomic level. In general the bacterial GAD system includes i) a glutamate uptake by a specific transporter followed by ii) the removal of an intracellular proton during glutamate decarboxylation and iii) GABA export from the cell via an antiporter. This leads to an increase in the cytoplasmic pH (by the removal of hydrogen ions) and also slightly increasing the extracellular pH (by the exchange of extracellular glutamate for GABA) (90). Interestingly, compared to the type strain DSM 20054, *Lb. brevis* FEM 1874 induces a higher expression of both *gadA* and *gadB* genes over time, accompanied by an increased level of *gadC*, the gene encoding for the antiporter and *gadR*, encoding for the positive operon regulator. Indeed, the higher activation of the GAD system observed could account for the high GABA production.

The physiological activity of GABA makes it an interesting bioactive molecule which has already been used as a food supplement in pure form (92). In recent years researchers have reported a number of placebo controlled studies in which GABA was administered as a food or oral supplement to healthy participants (26–31). GABA have been shown to be rapidly absorbed with the half-life of 5 h in a human intervention study where twelve healthy subjects were subjected to oral administration of 2 g GABA once or 2 g GABA three times/day for 7 days (31). A recent study has shown that the ingestion of 800 mg synthetic GABA enhanced the ability of prioritized planned actions (30). 10 gr of chocolate enriched with 28 mg GABA (27), a beverage containing 50 mg GABA (28), or 100 mg encapsulated GABA (29) were reported to reduce psychological fatigue and psychological stress after completion of an arithmetic task. To reach those doses, one would have to eat more than 2 kg of uncooked spinach, a vegetable rich in GABA (93). A pioneering study in patients with mild hypertension reported that daily intake of fermented milk containing 10-12 mg/100 mL of GABA could significantly lower blood pressure within 2 weeks (35). In these terms fermented milk enriched in GABA produced by *Lb. brevis* FEM 1874 may have commercial potential as a health-oriented dairy product as well as any direct probiotic effect of the high GABA producing strain.

To be considered a possible probiotic the bacterial strain, in addition to being a GRAS organism, should be able to survive within the human GI tract and therein mediate a specific health related activity in the right environment (68,69). Testing for tolerance of low pH, bile acids and pancreatic fluids have often been considered as good indicators for survival through the GI tract. In this study, *Lb. brevis* FEM 1874 strain performed well in the *in vitro* tests, and survival through the stomach is likely. In addition, previous studies have shown that food matrix plays an important role in probiotic survival of gastric pH (94,95) and cheeses in particular appears to effectively protect probiotics from low pH

encountered in the stomach (96,97). However, survival under in *vivo* conditions in human subjects should be tested.

Lb. brevis FEM 1874 was able to ferment and growth on several carbon sources including arabinogalactan, Arabinogalactan is a non-starch polysaccharide found in coffee beans, soybeans, broad beans, larch, tamarack, and cereals (98). Differently to what occurred with fructo-oligosaccharides, arabinogalactan acts as a prebiotic for the distal colon microbiota (99). Its oral administration has been reported to increase *Lactobacillus spp*. (100). Due to its saccharolytic function, short chain fatty acid production and ammonia level reduction (101) arabinogalactan could be used in the treatment of diseases characterized by ammonia build-up in the liver such as cirrhosis, chronic liver diseases and portal systemic encephalopathy. The potential action of arabinogalactan on the gut:brain:liver axis and the GABA producing capability of FEM 1874 suggest the possibility of using a combination of both in synbiotic formulation.

Lactic acid bacteria are intrinsically resistant to many antibiotics (102–109). In many cases resistances are not, however, transmissible, and the species are also sensitive to many clinically used antibiotics even in the case of a lactic acid bacteria- associated opportunistic infection. Among 187 isolates from 55 European probiotic products showed that 79% of the isolates were resistant against kanamycin and 65% of the isolates were vancomycin resistant. Remaining resistances were in the order of tetracycline (26%), penicillin G (23%), erythromycin (16%) and chloramphenicol (11%). Overall, 68.4% of the isolates showed resistance against multiple antibiotics including intrinsic resistance (110). The antimicrobial susceptibility tests indicated that *Lb. brevis* FEM 1874 was sensitive to ampicillin and mildly resistant toward gentamycin, clindamycin, erythromycin and vancomycin. To note that a *Lb. brevis* strain isolated from human GIT (103) has been shown to be resistant to higher level of vancomycin (256 mg/ml) and clindamycin (32 mg/ml) than *Lb. brevis* FEM 1874. Furthermore, the probiotic *L. brevis* KB290 MICs of tetracycline, and vancomycin were four, and eight times, respectively, higher than the breakpoint MICs suggested by the European Scientific Committee on Animal Nutrition, and the MIC of tetracycline was eight times the MIC suggested by the European Scientific Panel on Additives and Products or Substances Used in Animal Feed (109). The main concern amongst LAB is the resistance to vancomycin, within the genus *Enterococcus* where the resistance has been shown to be transferable (111). Vancomycin resistance is generally considered as an intrinsic property in lactobacilli, however, being described in several isolates from fermented food, diary and GIT (69,103–105,107–109) and this resistance also has been described in the widely used probiotic strain *Lb. rhamnosus* GG (108), where it has been described as not transferable. Therefore no particular safety concern is associated with this intrinsic type of resistance. Of course, we will investigate if the antibiotic resistances of FEM 1874 are intrinsic and we will perform plasmid curing of FEM 1874 to eliminate plasmid-associated antibiotic resistance, if any.

Even though *in vivo* investigations are needed, altogether these preliminary results showed that the *Lb. brevis* GABA producing FEM 1874 strain represents a promising

starter for manufacturing GABA-rich cultured dairy foods to be used as functional food as well as a promising next generation probiotic in the context of the gut(microbiota):brain axis. Tests on GABA level availability in FEM 1874 dairy products are ongoing. Overall our data indicate the importance of studying and preserving the traditional raw milk cheese microbiome. Traditional cheeses represent an important source of microbial biodiversity where new LAB strains with potential health promoting properties can be isolated.

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Supplementary Tables

Table S1. List of utilized primers for gad genes locus amplification.

Primer name	Primer sequence	PCR product size (bp)/target gene
gadR2-F	3'- AAACGGCTAGTTTTGGAAAGC -5'	408bp/*gadR*
gadR2-R	3'- AACGACCAAAGCCGGATTTT -5'	
gadR3-F	3'- AGAACTAAGGAAAGGCTGGGG -5'	218bp/*gadR*
gadR3-R	3'- TAACTAGCCAGCCAGTTGTCG -5'	
gadR4-F	3'- TCGATCTGATTGTGGAACGA -5'	208bp/*gadR*
gadR4-R	3'- TCTAATAACATGGCCAATTGC -5'	
gadR/intra_F	3'- CAGAGTCTGAAGCAGGCATGT -5'	549bp/intergenic region
gadR/intra_R	3'- AATGCCGCAAAACCGTAAAC -5'	
gadA4-F	3'- ACCACGCAAATGGAACCACAA -5'	894bp/*gadA*
gadA4-R	3'- CTTCAATGACACCTTCCGAA -5'	
gadA7-F	3'- CTTTGTGGTCATGCTCGTTTT -5'	576bp/*gadA*
gadA7-R	3'- CAGTTGAGGTCCCAATGAAA -5'	
gadA8-F	3'- TTTACCCGCAGAAATGCGAT -5'	711bp/*gadA*
gadA8-R	3'- ATGGTTCCGTGATAGTGCCG -5'	
gadIntra/gadA_F	3'- ATCCGTTGCCTCAAAACACA -5'	394bp/intergenic region
gadIntra/gadA_R	3'- CGATAGTGTTCCACCAATTGA -5'	
gad/anti1-F	3'- GATTGCCCAATGGTGTTTCA -5'	545bp/*gadC*
gad/anti1-R	3'- TCCCATATTTATTGGCCTTAGAG -5'	
gad/anti2-F	3'- TCCCAAATTGAAACCGCTGT -5'	737bp/*gadC*
gad/anti2-R	3'- ACCGGCAAAAGCCAAGATAA -5'	
gad/anti4-F	3'- TTTACGCCTATGGGGCCTT -5'	674bp/*gadC*
gad/anti4-R	3'- GGTTTCTTTTTCCAACGCCT -5'	
gadB4-F	3'- CGGTTATCAAGTTTGTTGGG -5'	458bp/*gadB*
gadB4-R	3'- AGGCACTGTGGGAGAAGTTGAT -5'	
gadB6-F	3'- ATCTTACTCCGGTCCCTTTGA -5'	662bp/*gadB*
gadB6-R	3'- GGTTGATGGGCAGTTAAGTCA -5'	
gadB7-F	3'- TAATCTGGCGTGACCAACA -5'	696bp/*gadB*

gadB7-R	3'- CAATGATGGTAAACGCCGAA -5'

Table S2. List of utilized primers for gad genes expression analysis in presence of 30 mg/ml of MSG.

Primer name	Primer sequence	PCR product size (bp)/target gene
gadA_RT2-F	3'- GCCAATTAATGGTGACCAAGT -5'	110bp/*gadA*
gadA_RT2-R	3'- CGGAGCCTGTGTACGTAATG -5'	
gadB_RT2-F	3'- GTCCTTGAATGTCGATCACG -5'	126bp/*gadB*
gadB_RT2-R	3'- CGCTCTACAACGGCATCTAA -5'	
gad/anti_RT-F	3'- AAGATTGCCCAATGGTGTTT -5'	147bp/*gadC*
gad/anti_RT-R	3'- ACTCCCATTCCAACTCGATG -5'	
gad/R_RT1-F	3'- CCCATGCTTATTCGGAATTT -5'	111bp/*gadR*
gadR_RT1-R	3'- CATTGCGGAAATGTAACTGC -5'	
Lb_tuf2-F	3'- GCCGCTCAAATGGACGGTGC -5'	230bp/*tuf1*
Lb_tuf2-R	3'- AGCTGAACCGCGGATAACAGGA -5'	

Conclusions

There are many potential mechanisms by which gut microbiota can influence human (patho)physiology. Indeed recent fascinating evidence has emerged on the deep interconnections between the host central nervous system, brain health and gut microbiota. Alterations in the gut:brain axis have been implicated in the pathogenesis of gut disorders such as irritable bowel syndrome and related functional gastro intestinal disorders and also in several psychiatric conditions including autism spectrum disorders, depression and chronic pain. In most of these disorders a shift from the healthy symbiotic gut microbiota to a dysbiotic condition is repeated and seems to represent the turning point in the evolution of pathogenesis or at least the onset of these diseases. Dysbiotic microbiota has also been reported in liver diseases and their complications such as hepatic encephalopathy (HE). This debilitating complication of liver failure may be considered as a clear example of how an altered gut microbiota homeostatis can influence physiological function outside the intestine with implication for host health at the systems level.

The studies presented in this thesis uses a multilevel approach to study i) the microbial dynamics and metabolic activity of the liver disease microbiota, upon modulation by prebiotic, antibiotic and probiotic; ii) the impact of the probiotic VSL#3 on the gut microbiota of children with HE in double blind, randomized pilot scale dietary intervention run by the U.S.S.D Epatologia Gastroenterologia e Trapianti pediatrici (Ospedale di Bergamo); iii) initial probiotic strain characterization of *Lactobacillus brevis* FEM 1874 strain, selected as a putative next generation probiotic capable of impacting on host physiology through the production of the neurotransmitter γ-amino-butyric acid (GABA).

Measuring cirrhotic microbiota modulation by prebiotic, antibiotic and probiotic treatment using *in vitro* faecal batch cultures

Gut microbiota alteration represents a key factor in cirrhosis progression and appear to be related to neuropsychiatric complications. In particular, microbial dysbiosis and gut ammonia production by microbial activities are the main factors implicated in HE development. Most of the available HE therapies concentrate on reducing serum ammonia levels by decreasing its production in the intestine and increasing its elimination. Despite apparent clinical efficacy of prebiotics, antibiotics and probiotics to ameliorate mental and cognitive status in HE by reducing ammonia levels, little is known about the dynamics, interactions and bacteria, responsible for metabolite production within the cirrhotic gut microbiota. Moreover, data on ammonia levels are usually related to circulating levels and not based on ammonia concentrations within the colonic environment. Here, I investigated how cirrhotic microbiota is modulated by lactulose, rifaximin and VSL#3, treatments currently used in clinical practice in the treatment of HE. I used *in vitro* pH controlled batch cultures using faecal samples from 10 cirrhotic patients to evaluate changes in the microbiota structure, short fatty acid production and ammonia concentration. Data from this *in vitro* approach showed how the microbial environment characteristic of cirrhosis can be modulated dynamically at both the community structural and metabolic levels. Although lactulose, VSL#3, rifaximin and their mixtures all appeared to modulate the cirrhotic microbiota to some degree changing relative abundance of certain bacterial taxa, their major impact appeared to be at the metabolic level.

Across the 24 hour of fermentation, data showed that at least 10 hours are needed to induce the most appreciable changes. In particular, few changes have been observed at the population structural level, where rifaximin fermentation leads to the larger modulation in terms of taxa relative abundance. After the 24 hours fermentation, rifaximin or its association with lactulose or lactulose plus VSL#3 significantly decreased the abundance of Clostriadiales, Lachnospiraceae, Vellonellaceae and at genus level, *Blautia* abundance in agreement with a previous study on the mucosal microbiota composition of HE patients supplemented with rifaximin plus lactulose. Moreover, rifaximin alone and in combination with lactulose was able to strongly reduce Streptococcaceae relative abundance which were previously associated to poor cognitive performance in HE patients. Furthermore, in response to rifaximin we observed a decrease of *Collinsella*, which was found overabudant in mice with NAFLD induced by a high fat diet. I observed a particularly large increase in bifidobacteria beneficial group in lactulose fermentation. Presence of the prebiotic was also associated with acetate, propionate and butyrate production, and reduced concentration of ammonia. This shift in metabolite production is indicative of carbohydrate fermentation that could also significantly increased consumption or conversion of ammonia and other nitrogenous compounds in bacterial biomass. In either case, reduced ammonia concentrations and increased concentration of SCFA are consistent with improved gut health and reduced risk of HE.

Previously, human intervention studies have usually only shown a clear association between cognitive performance, liver disease, gut microbiota and ammonia increase and not causation. Co-occurance was observed between certain microbial changes and

improving symptoms. Although *in vitro* batch cultures provide a technically simple way of studying complex biological systems, here, they helped to correlate ecological niches and metabolic activities with particular phylogenetic groups amongst the gut microbiota of cirrhotic patients. The results emphasize the importance of prebiotic in shifting fermentation patterns of the cirrhotic microbiota towards SCFA production while reducing ammonia level and also the possible synergistic effects of lactulose, VSL#3 and rifaximin in lowering colonic ammonia accumulation and possibly HE symptomatology. However, any synergistic affect awaits validation in suitably designed human intervention studies in HE patients.

Effect of VSL#3 treatment in paediatric patients and young adults affected by Minimal Hepatic Encephalopathy from portal hypertension: a pilot intervention study

Many liver and vascular diseases cause portal vein hypertension in children. Portal vein hypertension may give rise to severe and life-threatening complications, including haemorrhaging from oesophageal varices, ascites, hepatopulmonary syndrome, portopulmonary hypertension and HE. The effective prevention and management of portal hypertension and its complications, therefore, are important goals for improving health related outcomes and quality of life in affected children. Prebiotics, antibiotics, L-ornithine L-aspartate, branched aminoacids, probiotics and synbiotics as well as a low protein diet have all been shown to improve psychometric performance and quality of life in this disease group.

Generally defined as “life microorganisms that produce a beneficial effect to the host when administered in an adequate amount”, probiotics have been shown to be effective in amelioration of liver disease conditions by (i) changing gut metabolism; (ii) reducing ammonia in the portal blood (decreasing bacterial urease activity, intestinal permeability, ammonia absorption by lowering pH and improving nutritional status of the gut epithelium); (iii) reducing hepatic inflammation and oxidative stress; (iv) reducing the absorption of other toxins such as indoles, oxindoles, phenols and mercaptans. However, despite their potential, most studies evaluating probiotics in experimental models of cirrhosis and portal hypertension have given conflicting results. Nevertheless, several human intervention studies, mainly in the field of HE, have provided supportive evidence for the efficacy of certain probiotics in the improvement of Minimal HE (MHE), in the prevention of HE recurrence and prophylaxis of Overt HE.

Here, I presented a pilot intervention study in paediatric and young adults affected by portal vein hypertension and manifesting with symptoms of MHE (https://clinicaltrials.gov/ct2/show/NCT01798329). Intervention was with VSL#3 or placebo for 3 months. The study was initially powered at 25 patients per group but because of difficulties in recruiting patients meeting the inclusion and exclusion criteria the

responsible clinicians were only able to provide faecal samples from 8 patients in the VSL#3 treated group and 6 patients in the placebo group. I performed 16S rRNA sequencing on faecal samples before and after the treatment from patients involved in this pilot study. The overall aim was to study the effect of VSL#3 in ameliorating cognitive function and patients quality of life by modulating the gut microbiota and reducing the ammonia level. Despite the small sample size VSL#3 supplementation resulted in a trend towards improved cognitive function but not a significant change in the gut microbiota nor a decrease in blood ammonia levels. Even though not statistically significant a trend towards an increased relative abundance in Actinobacteria and a concomitant decrease in Bacteroidetes was evident from the 16S rRNA profiling data. The results suggested also a mild decrease in *Bacteroides* relative abundance, as well as a slight increase of *Ruminococcus* and *Faecalibacterium*. *Ruminococcus* has been previously associated with secondary bile acid production and decreased severity of cirrhosis progression.

This pilot intervention study suffered from the small number of subjects recruited (14 out of 50 expected) and potential of the different underlying pathological causes of portal vein hypertension to influence baseline variation in gut microbiota composition. Moreover, VSL#3 might induce different alterations in the microbiota from different patients that are probably not consistent between subjects, due to their intrinsic difference in microbiota composition. A larger study with a more potent stratification for different underlying liver disease is needed to prove the link between gut microbiota changes in terms of community structure and metabolism and the efficacy of VSL#3 in ameliorating the disease condition. However, according to previous studies in adults, the 50 patients we initially planned to enrol might be sufficient to observe microbiota changes as well as changes in the plasma ammonia or transaminase levels.

Conflicting results directly linking the dysbiosis and the improvement of cognitive function in HE therapies have also been observed in response to other clinical therapies such as lactulose or rifaximin. Moreover, also my previous investigation using *in vitro* faecal batch cultures showed only modest changes of bacterial community structure upon VSL#3 fermentation. This suggests that further investigations should not be restricted to measuring the relative abundance of particular bacterial taxa but should also evaluate any changes in the gut microbiota metabolism. Indeed, 16S rRNA profiling flanked by a metabolomic approach would allow better understanding of the link between microbiota modulation and disease symptoms.

Probiotic characterization of high GABA producing strain *Lactobacillus brevis* FEM 1874

Probiotic interventions targeting the microbiota-gut-brain axis to modulate behavior have recently been reported. *Lb. rhamnosus* JB-1 promoted an anxiolytic–antidepressant-like effect through alterations in the expression of GABA receptors, such as

such as GABAAα2, GABAAα1, and GABAB1b. Indeed, GABA receptors targeting represents a goal for improving brain function. Identification of bacteria able to produce high GABA levels and bearing features of a probiotic is a viable approach for designing efficacious next generation probiotics targeting the gut:brain axis. A previous study of our group isolated 276 strains from a specific raw cow milk "Nostrano-cheese", typical of the Trentino province (north, alpine area) in Italy. 71% bacterial strains were able to produce GABA. Among those, *Lb. brevis* FEM 1874 possessed the highest glutamate decarboxylase (GAD) activity compared to the type strain. We therefore sought to characterize its potential as a novel next generation probiotic. Indeed the identification of strains with specific mode of action or biochemical trait capable of mediating specific host physiological responses, represents the basis of a rational scientific selection of next generation probiotic strains designed to mediate specific health effects in the host. Targets will include microbiota-impacted physiological functions extending beyond the gut.

Lb. brevis FEM 1874 accumulated high levels of GABA in the culture medium thanks to a higher transcriptional GAD activity compared to the type strain. FEM 1874 was able to efficiently convert glutamate to GABA by the increased expression of the operon regulator (*gadR*) and the (*gadC*) antiporter encoding genes. Testing for tolerance of low pH, bile acids and pancreatic fluids have often been considered as good indicators for survival through the GI tract. In this study, *Lb. brevis* FEM 1874 strain proved resistant to low pH, bile acids and pancreatic fluids thus suggesting its survival through the stomach is likely. However, survival under in *vivo* conditions in human subjects should be tested.

There are some concerns about the ability of free GABA in reaching the brain and cross the brain barrier. However, the physiological activity of GABA makes it an interesting bioactive molecule which has already been used as a food supplement in pure form. GABA was shown to reduce blood pressure and heart rate and anxiety as well as to have anti-proliferative activity on colon carcinoma cells. Moreover GABA supplementation resulted in inflammation relief by decreasing cytokine secretion and T cells proliferation. In these terms fermented milk enriched in GABA produced by *Lb. brevis* FEM 1874 may have commercial potential as a health-oriented dairy product as well as any direct probiotic effect of the high GABA producing strain. Tests on GABA level availability in FEM 1874 dairy products are ongoing. My data showed that *Lb. brevis* FEM 1874 was able to ferment and growth on arabinogalactan which oral administration has been reported to increase *Lactobacillus spp*., to promote short chain fatty acid production and ammonia level reduction. The potential action of arabinogalactan on the gut:brain:liver axis and the GABA producing capability of FEM 1874 suggest the possibility of using a combination of both in synbiotic formulation.

Overall, this preliminary characterization indicates the potential of FEM 1874 as a next-generation probiotic targeting disruptions occurring in the gut(microbiota):brain axis, as in the case of advanced liver disease and liver failure as well as systemic inflammation through GABA production.

To conclude, this thesis work showed the efficacious of prebiotic, probiotic and antibiotic in targeting the dysbiosis related to gut:liver:brain axis disruption, as in the case of cirrhosis and HE. The major changes occurred at the metabolic level with a reduction in ammonia accumulation and production of SCFA, especially when a synbiotic formulation is used. Of consequence this restored metabolic make up will account for ameliorating clinical symptoms and quality of life of cirrhotic and HE patients. The research moved also a step forward by identifying a *Lb. brevis* strain capable of producing and secreting high amount of GABA. *Lb. brevis* FEM 1874 might be a promising probiotic or a starter for dairy fermentation to manufacture GABA-rich cultured dairy foods to be used in restoring or ameliorating conditions linked to an altered gut :liver:brain axis.

Appendix A

Supplementary Tables Chapter 2

Table A1. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at phylum order and family levels in Healthy subjects (HS), Cirrhotic patients (CP) and CP subgroups at the baseline. ALC, alcohol cirrhosis; AI, autoimmune cirrhosis; NASH, non-alcoholic stehatosis.

	Health status									
Phylum	**HS**		**CP**		**ALC**		**AI**		**NASH**	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinobacteria	6.281	8.433	4.202	3.389	3.858	2.579	3.645	2.644	4.741	4.030
Bacteroidetes	31.33	12.64	34.19	10.58	34.22	10.41	39.83	9.60	34.27	11.00
Chloroflexi	0.000	0.000	0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.000
Cyanobacteria	0.007	0.011	0.016	0.039	0.005	0.009	0.058	0.079	0.017	0.041
Euryarchaeota	0.014	0.042	0.007	0.028	0.005	0.017	0.000	0.000	0.015	0.035
Firmicutes	56.753	12.584	56.405	9.089	56.754	8.810	54.357	9.163	57.082	9.520
Fusobacteria	0.031	0.095	0.060	0.172	0.057	0.162	0.000	0.000	0.067	0.196
Lentisphaerae	0.004	0.009	0.004	0.009	0.003	0.007	0.007	0.013	0.003	0.010
Proteobacteria	4.786	6.458	3.375	6.095	4.246	7.036	1.258	0.439	2.942	5.765
Synergistetes	0.000	0.002	0.003	0.015	0.001	0.007	0.000	0.000	0.007	0.020
Tenericutes	0.006	0.019	0.010	0.027	0.016	0.035	0.000	0.000	0.005	0.022
TM7	0.000	0.000	0.002	0.005	0.003	0.006	0.005	0.005	0.001	0.002
Verrucomicrobia	0.013	0.020	0.039	0.103	0.026	0.043	0.017	0.016	0.072	0.137
Other	0.774	0.332	0.776	0.285	0.798	0.296	0.815	0.391	0.772	0.260

	Health status									
Order	**HS**		**CP**		**ALC**		**AI**		**NASH**	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinomycetales	0.01	0.01	0.025	0.023	0.027	0.022	0.024	0.020	0.022	0.024
Aeromonadales	0.00	0.00	0.001	0.004	0.001	0.004	0.00	0.00	0.001	0.004
Anaerolineales	0.00	0.00	0.000	0.001	0.000	0.002	0.00	0.00	0.000	0.000
Bacillales	0.00	0.00	0.001	0.004	0.002	0.005	0.00	0.00	0.001	0.002
Bacteroidales	31.5	12.7	34.87	10.66	34.49	10.50	38.6	9.65	34.50	11.09
Bifidobacteriales	4.09	6.11	3.535	3.410	3.133	2.624	2.60	2.39	4.031	4.061
Burkholderiales	1.10	1.17	0.911	0.423	0.819	0.317	0.76	0.22	1.013	0.502
Campylobacterales	0.00	0.00	0.006	0.014	0.009	0.015	0.00	0.00	0.004	0.014
Cardiobacteriales	0.00	0.00	0.000	0.002	0.001	0.002	0.00	0.00	0.000	0.002
Clostridiales	40.69	10.9	39.25	12.39	38.70	14.55	48.9	10.3	37.97	10.13
Coriobacteriales	2.22	6.07	0.734	0.519	0.731	0.538	0.83	0.35	0.718	0.538
CW040	0.00	0.00	0.000	0.003	0.001	0.004	0.00	0.00	0.000	0.000
Desulfovibrionales	0.60	0.58	0.199	0.182	0.132	0.103	0.42	0.25	0.214	0.188

Enterobacteriales	3.08	5.44	2.160	6.009	3.199	6.931	0.09	0.05	1.678	5.661
Erysipelotrichales	7.80	13.7	1.417	1.157	1.241	1.228	0.88	0.37	1.657	1.150
Flavobacteriales	0.00	0.00	0.000	0.001	0.000	0.000	0.00	0.00	0.000	0.002
Fusobacteriales	0.03	0.09	0.058	0.174	0.057	0.163	0.00	0.00	0.068	0.198
Gemellales	0.00	0.00	0.008	0.016	0.006	0.012	0.00	0.01	0.010	0.019
I025	0.00	0.00	0.000	0.001	0.000	0.000	0.00	0.00	0.000	0.000
Lactobacillales	8.68	8.79	16.54	14.77	17.249	16.864	6.605	8.830	17.742	13.339
Methanobacteriales	0.014	0.042	0.009	0.028	0.005	0.018	0.000	0.000	0.015	0.035
ML615J-28	0.004	0.017	0.006	0.022	0.012	0.029	0.000	0.000	0.003	0.016
Neisseriales	0.000	0.000	0.000	0.002	0.001	0.002	0.000	0.000	0.000	0.002
Pasteurellales	0.011	0.022	0.068	0.166	0.102	0.202	0.009	0.011	0.051	0.143
Pseudomonadales	0.000	0.002	0.000	0.003	0.001	0.004	0.000	0.000	0.000	0.000
RF32	0.011	0.018	0.009	0.020	0.016	0.025	0.004	0.008	0.004	0.013
RF39	0.003	0.005	0.003	0.009	0.004	0.011	0.000	0.000	0.003	0.009
Rhizobiales	0.002	0.004	0.002	0.004	0.002	0.004	0.001	0.004	0.001	0.004
SHA-98	0.000	0.002	0.002	0.006	0.003	0.009	0.001	0.004	0.001	0.004
Streptophyta	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.001	0.004
Synergistales	0.000	0.002	0.004	0.015	0.001	0.007	0.000	0.000	0.007	0.020
Turicibacterales	0.030	0.068	0.044	0.090	0.012	0.053	0.056	0.073	0.069	0.109
Verrucomicrobiales	0.013	0.020	0.048	0.104	0.026	0.043	0.014	0.016	0.072	0.138
Victivallales	0.004	0.009	0.004	0.009	0.003	0.007	0.010	0.013	0.003	0.010
YS2	0.005	0.010	0.015	0.040	0.005	0.010	0.050	0.080	0.017	0.043

	Health status									
Family	**CP** mean ± sd		**HS** mean ± sd		**ALC** mean ± sd		**AI** mean ± sd		**NASH** mean ± sd	
Actinomycetaceae	0.017	0.020	0.010	0.01	0.020	0.019	0.015	0.015	0.015	0.015
Aerococcaceae	0.001	0.003	0.000	0.00	0.001	0.001	0.000	0.000	0.001	0.001
Alcaligenaceae	0.944	0.443	1.141	1.19	0.847	0.837	0.821	0.824	0.987	0.974
Bacteroidaceae	25.70	12.28	25.95	11.8	28.39	28.40	27.64	27.85	23.42	23.73
Bifidobacteriaceae	3.675	3.539	4.237	6.25	3.234	3.253	2.839	2.871	4.128	3.869
Burkholderiaceae	0.000	0.002	0.000	0.00	0.001	0.000	0.000	0.000	0.000	0.000
Campylobacteraceae	0.002	0.006	0.000	0.00	0.004	0.003	0.002	0.002	0.001	0.001
Carnobacteriaceae	0.012	0.019	0.006	0.01	0.007	0.007	0.011	0.012	0.015	0.016
Christensenellaceae	0.059	0.157	0.077	0.15	0.101	0.104	0.114	0.103	0.036	0.037
Clostridiaceae	0.684	0.901	0.808	1.12	0.433	0.416	0.506	0.520	0.910	0.843
Coriobacteriaceae	0.769	0.550	2.283	6.15	0.766	0.785	0.940	0.948	0.703	0.717
Corynebacteriaceae	0.001	0.004	0.000	0.00	0.002	0.002	0.001	0.001	0.000	0.000

Dehalobacteriaceae	0.003	0.008	0.001	0.07	0.005	0.005	0.005	0.005	0.002	0.002
Desulfovibrionaceae	0.209	0.195	0.630	0.59	0.138	0.142	0.226	0.229	0.226	0.219
Dethiosulfovibrionaceae	0.004	0.016	0.000	0.07	0.001	0.001	0.001	0.001	0.004	0.004
Enterobacteriaceae	2.205	6.124	3.155	5.55	3.265	2.728	1.230	1.261	1.937	1.992
Enterococcaceae	0.300	0.663	0.644	2.00	0.522	0.497	0.461	0.469	0.198	0.203
Erysipelotrichaceae	1.481	1.234	7.976	13.9	1.298	1.270	1.311	1.337	1.670	1.702
Eubacteriaceae	0.002	0.005	0.001	0.00	0.002	0.002	0.002	0.002	0.001	0.001
Fusobacteriaceae	0.058	0.176	0.031	0.09	0.058	0.060	0.005	0.005	0.083	0.085
Gemellaceae	0.008	0.016	0.001	0.00	0.006	0.005	0.005	0.005	0.012	0.012
Helicobacteraceae	0.004	0.014	0.001	0.00	0.006	0.006	0.006	0.006	0.004	0.004
Lachnospiraceae	17.96	5.659	17.51	6.69	17.72	17.67	20.55	20.45	17.28	17.51
Lactobacillaceae	4.084	4.586	1.375	1.61	3.575	3.685	2.206	2.198	4.877	4.791
Leuconostocaceae	0.014	0.034	0.021	0.04	0.022	0.022	0.022	0.022	0.006	0.006
Methanobacteriaceae	0.010	0.029	0.015	0.04	0.005	0.005	0.005	0.005	0.009	0.009
Microbacteriaceae	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Micrococcaceae	0.007	0.012	0.000	0.00	0.006	0.006	0.008	0.008	0.007	0.007
Neisseriaceae	0.000	0.002	0.000	0.00	0.001	0.001	0.000	0.000	0.000	0.000
Oxalobacteraceae	0.001	0.004	0.000	0.00	0.001	0.001	0.001	0.001	0.001	0.001
Pasteurellaceae	0.070	0.169	0.011	0.02	0.105	0.087	0.046	0.047	0.055	0.056
Peptococcaceae	0.016	0.079	0.038	0.11	0.022	0.023	0.025	0.026	0.017	0.017
Peptostreptococcaceae	0.014	0.021	0.014	0.03	0.011	0.011	0.006	0.007	0.018	0.017
Phyllobacteriaceae	0.002	0.004	0.002	0.00	0.002	0.002	0.002	0.002	0.002	0.002
Porphyromonadaceae	1.219	1.073	1.534	1.06	1.162	1.199	1.508	1.520	1.178	1.215
Prevotellaceae	5.500	11.454	1.372	2.15	2.651	2.730	3.891	4.012	7.460	7.692
Propionibacteriaceae	0.000	0.002	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
Pseudomonadaceae	0.000	0.003	0.000	0.00	0.001	0.001	0.001	0.001	0.000	0.000
Rikenellaceae	1.335	1.364	1.460	1.43	1.525	1.546	1.829	1.767	1.196	1.219
Ruminococcaceae	16.343	7.355	17.980	8.21	16.535	16.603	19.514	19.464	15.810	15.803
S24-7	0.897	2.961	1.597	3.81	1.187	1.224	2.068	2.132	0.181	0.187
Staphylococcaceae	0.001	0.003	0.000	0.00	0.001	0.001	0.001	0.001	0.001	0.001
Streptococcaceae	12.58	12.51	6.944	7.71	13.46	13.76	8.909	8.558	13.14	12.55
Succinivibrionaceae	0.001	0.004	0.000	0.00	0.001	0.001	0.000	0.000	0.001	0.001
Turicibacteraceae	0.047	0.096	0.032	0.07	0.013	0.013	0.025	0.026	0.077	0.072
Veillonellaceae	2.151	1.275	1.880	1.29	1.840	1.793	1.726	1.756	2.422	2.460
Verrucomicrobiaceae	0.050	0.108	0.014	0.02	0.027	0.028	0.031	0.032	0.049	0.050
Victivallaceae	0.004	0.010	0.004	0.01	0.003	0.003	0.006	0.006	0.004	0.004

[Barnesiellaceae]	0.334	0.358	0.349	0.40	0.307	0.312	0.381	0.389	0.287	0.296
[Cerasicoccaceae]	0.000	0.001	0.000	0.00	0.000	0.000	0.000	0.000	0.000	0.000
[Mogibacteriaceae]	0.084	0.106	0.172	0.23	0.105	0.108	0.126	0.124	0.070	0.072
[Odoribacteraceae]	0.311	0.237	0.399	0.26	0.357	0.365	0.438	0.431	0.246	0.252
[Paraprevotellaceae]	0.818	1.492	0.311	0.70	0.235	0.243	0.520	0.536	1.228	1.266
[Tissierellaceae]	0.001	0.004	0.003	0.00	0.002	0.001	0.001	0.001	0.002	0.002

Table A2. Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial microbiota on the unweighted and weighted UniFrac distances and the Bray-Curtis dissimilarity according to treatments and time points. ctrl, Control; LR, Lactulose+Rifaximin; R, Rifaximin; V, VSL#3; VL, VSL#3+Lactulose; VLR, VSL#3+Lactulose+Rifaximin; VR, VSL#3+Rifaximin

		Time 0			Time 5			Time 10			Time 24		
Treatment	**Metric**	***F***	R^2	**p-value***	***F***	R^2	**p-value**	***F***	R^2	**p-value**	***F***	R^2	**p-value**
ctrl	Unweighted UniFrac	0.4732	0.0412	0.7030	0.3444	0.0304	0.8480	0.1286	0.0116	0.9810	0.6847	0.0586	0.5620
	Weighted UniFrac	1.0267	0.0854	0.3360	1.006	0.0839	0.4180	1.0032	0.0836	0.4110	0.8930	0.0751	0.9530
	Bray-Curtis	1.3084	0.1063	0.1530	0.9531	0.0797	0.4850	0.8777	0.0739	0.6790	0.8326	0.0704	0.7130
L	Unweighted UniFrac	0.3009	0.0266	0.7910	0.9448	0.0791	0.4260	0.3785	0.0333	0.8490	0.1984	0.0177	0.9650
	Weighted UniFrac	1.022	0.0851	0.3460	1.1041	0.0912	0.0850	0.9177	0.0770	0.7790	0.9722	0.0812	0.5190
	Bray-Curtis	1.6511	0.1305	0.0520	0.9481	0.0794	0.5230	0.7518	0.0640	0.7630	1.104	0.0913	0.2920
LR	Unweighted UniFrac	2.5800	0.1900	0.0810	0.3519	0.0310	0.8050	0.0221	0.0020	0.9830	0.6791	0.0581	0.5100
	Weighted UniFrac	1.0955	0.0906	0.1730	0.9615	0.0804	0.6600	0.9473	0.0793	0.6920	1.2054	0.0988	0.0490
	Bray-Curtis	1.2903	0.1050	0.1540	0.9225	0.0774	0.5870	1.3092	0.1064	0.2410	1.0909	0.0902	0.2850
R	Unweighted UniFrac	0.3243	0.0286	0.8370	0.6257	0.0538	0.5220	1.4125	0.1138	0.2000	0.2372	0.0211	0.9370
	Weighted UniFrac	0.9704	0.0811	0.5640	0.9185	0.0771	0.7530	1.0024	0.0835	0.4240	0.8931	0.0751	0.7750
	Bray-Curtis	0.8443	0.0713	0.6670	0.7209	0.0615	0.7590	0.7583	0.0645	0.8550	0.5648	0.0488	0.9140
V	Unweighted UniFrac	2.0535	0.1573	0.0900	0.9124	0.0766	0.4180	0.9991	0.0833	0.3460	0.4798	0.0418	0.7140
	Weighted UniFrac	1.2022	0.0985	0.0630	1.0283	0.0855	0.2880	0.9995	0.0833	0.4360	1.0656	0.0883	0.2030
	Bray-Curtis	1.3574	0.1098	0.1280	1.2983	0.1056	0.1190	0.8895	0.0748	0.6040	1.0768	0.0892	0.3420
VL	Unweighted UniFrac	1.3995	0.1129	0.2580	1.1774	0.0967	0.3080	1.2584	0.1027	0.3410	3.5460	0.2438	*0.0210*
	Weighted UniFrac	0.9997	0.0833	0.4420	1.0097	0.0841	0.3890	1.0357	0.0861	0.3200	1.1524	0.0948	*0.0280*
	Bray-Curtis	0.9641	0.0806	0.4870	1.0356	0.0860	0.4050	0.8235	0.0697	0.6500	1.5578	0.1241	*0.0310*
VLR	Unweighted UniFrac	1.6493	0.1304	0.1190	0.3067	0.0271	0.7660	0.5237	0.0454	0.7380	1.3211	0.1072	0.3570
	Weighted UniFrac	1.2770	0.1040	0.0280	0.9594	0.0802	0.6310	0.9217	0.0773	0.8150	1.0771	0.0892	0.1460
	Bray-Curtis	2.1453	0.1632	*0.0150*	1.094	0.0905	0.2970	0.5868	0.0506	0.9520	0.9119	0.0766	0.6460
VR	Unweighted UniFrac	1.4753	0.1183	0.2810	0.9218	0.0773	0.5040	1.0724	0.0888	0.3670	1.1145	0.0920	0.3210
	Weighted UniFrac	0.9858	0.0823	0.4730	0.8799	0.0741	0.9770	1.0473	0.0869	0.2300	0.9978	0.0832	0.5130
	Bray-Curtis	0.8468	0.0715	0.6800	0.6802	0.0582	0.8860	1.3774	0.1113	0.0960	0.9622	0.0804	0.4490

*Bonferroni corrected p-values

Table A3. Mean relative abundance (%) ± standard deviation (sd) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) at the baseline, over time.

Phylum	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinobacteria	3.792	4.069	4.621	5.688	5.862	6.929	8.679	11.110
Bacteroidetes	36.543	9.436	32.665	8.634	22.225	16.252	31.624	6.892
Cyanobacteria	0.013	0.026	0.003	0.007	0.001	0.003	0.004	0.010
Euryarchaeota	0.016	0.051	0.012	0.038	0.021	0.045	0.045	0.099
Firmicutes	55.723	7.797	47.189	9.678	59.511	21.146	49.622	7.471
Fusobacteria	0.052	0.162	2.081	4.323	1.406	2.920	0.669	1.634
Lentisphaerae	0.006	0.013	0.004	0.009	0.004	0.008	0.006	0.016
Proteobacteria	3.779	6.367	13.351	7.327	10.900	10.947	9.305	7.771
Synergistetes	0.005	0.016	0.008	0.025	0.005	0.016	0.005	0.016
Tenericutes	0.012	0.038	0.002	0.004	0.000	0.000	0.000	0.000
TM7	0.003	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Verrucomicrobia	0.055	0.127	0.063	0.142	0.065	0.148	0.039	0.084

Order	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinomycetales	0.028	0.022	0.018	0.016	0.008	0.008	0.006	0.010
Aeromonadales	0.000	0.000	0.001	0.003	0.000	0.000	0.001	0.003
Bacillales	0.001	0.003	0.000	0.000	0.001	0.003	0.001	0.003
Bacteroidales	36.543	9.435	32.668	8.635	22.226	16.252	31.626	6.892
Bifidobacteriales	3.020	4.053	3.722	5.947	4.525	6.947	6.813	9.922
Burkholderiales	1.123	0.460	3.010	1.534	2.101	1.552	1.836	0.838
Campylobacterales	0.006	0.011	0.003	0.005	0.006	0.013	0.002	0.006
Cardiobacteriales	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Clostridiales	47.312	11.954	37.297	9.075	30.803	13.628	39.549	8.067
Coriobacteriales	0.745	0.499	0.881	0.458	1.329	2.300	1.860	1.573
CW040	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Desulfovibrionales	0.225	0.215	0.836	0.488	1.049	0.968	1.472	1.051
Enterobacteriales	2.320	6.207	9.430	8.144	7.675	9.564	5.967	6.836
Erysipelotrichales	1.307	0.883	2.853	4.021	24.279	29.884	4.310	7.531
Fusobacteriales	0.052	0.162	2.081	4.323	1.406	2.920	0.670	1.634
Gemellales	0.013	0.024	0.005	0.007	0.002	0.004	0.001	0.003
Lactobacillales	7.030	8.137	6.959	7.065	4.399	4.668	5.753	4.708
Methanobacteriales	0.016	0.051	0.012	0.038	0.021	0.045	0.045	0.099

ML615J-28	0.009	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Neisseriales	0.000	0.000	0.003	0.010	0.003	0.007	0.000	0.000
Pasteurellales	0.089	0.200	0.059	0.106	0.059	0.124	0.018	0.037
Pseudomonadales	0.000	0.000	0.001	0.003	0.000	0.000	0.001	0.003
RF32	0.015	0.026	0.004	0.013	0.004	0.013	0.001	0.003
RF39	0.003	0.010	0.002	0.004	0.000	0.000	0.000	0.000
Rhizobiales	0.000	0.000	0.000	0.000	0.002	0.004	0.002	0.004
SHA-98	0.003	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Sphingobacteriales	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sphingomonadales	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Streptophyta	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Synergistales	0.005	0.016	0.008	0.025	0.005	0.016	0.005	0.016
Turicibacterales	0.057	0.115	0.077	0.179	0.028	0.040	0.011	0.018
Verrucomicrobiales	0.055	0.127	0.062	0.139	0.065	0.148	0.039	0.084
Victivallales	0.006	0.013	0.004	0.009	0.004	0.008	0.006	0.016
YS2	0.013	0.026	0.003	0.007	0.001	0.003	0.004	0.010

Family	**Time point**							
	T0		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.383	0.392	0.457	0.435	0.281	0.429	0.523	0.535
[Mogibacteriaceae]	0.086	0.112	0.074	0.122	0.077	0.143	0.207	0.328
[Odoribacteraceae]	0.352	0.238	0.294	0.158	0.151	0.122	0.260	0.165
[Paraprevotellaceae]	0.924	1.822	0.305	0.434	0.573	1.353	0.142	0.260
[Tissierellaceae]	0.002	0.004	0.008	0.013	0.013	0.022	0.021	0.044
Actinomycetaceae	0.026	0.025	0.016	0.014	0.007	0.007	0.005	0.010
Aerococcaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Alcaligenaceae	1.172	0.479	3.083	1.541	2.133	1.564	1.904	0.850
Bacteroidaceae	28.14	12.63	26.87	11.30	19.89	15.61	24.09	9.845
Bifidobacteriaceae	3.183	4.313	3.805	6.028	4.575	6.997	7.120	10.324
Burkholderiaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Campylobacteraceae	0.004	0.010	0.003	0.005	0.006	0.013	0.002	0.007
Cardiobacteriaceae	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Carnobacteriaceae	0.018	0.015	0.007	0.009	0.002	0.006	0.002	0.004
Christensenellaceae	0.068	0.179	0.015	0.014	0.015	0.020	0.045	0.048
Clostridiaceae	0.838	1.225	1.975	4.013	1.082	2.033	0.402	0.439
Comamonadaceae	0.000	0.000	0.000	0.000	0.002	0.004	0.000	0.000
Coriobacteriaceae	0.781	0.522	0.909	0.469	1.346	2.308	1.947	1.678

Corynebacteriaceae	0.000	0.000	0.001	0.003	0.001	0.003	0.000	0.000
Dehalobacteriaceae	0.005	0.011	0.000	0.000	0.000	0.000	0.000	0.000
Desulfovibrionaceae	0.239	0.234	0.863	0.499	1.082	1.024	1.526	1.081
Dethiosulfovibrionaceae	0.005	0.017	0.008	0.026	0.005	0.016	0.005	0.017
Enterobacteriaceae	2.385	6.363	9.830	8.562	7.843	9.681	6.208	7.077
Enterococcaceae	0.442	0.841	0.305	0.699	0.295	0.602	1.007	2.281
Erysipelotrichaceae	1.361	0.911	2.922	4.067	24.510	30.176	4.485	7.806
Eubacteriaceae	0.003	0.007	0.001	0.003	0.004	0.007	0.003	0.007
Fusobacteriaceae	0.053	0.164	2.097	4.354	1.416	2.941	0.674	1.645
Gemellaceae	0.013	0.024	0.005	0.007	0.002	0.004	0.001	0.003
Helicobacteraceae	0.002	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	22.403	5.215	13.390	3.862	12.961	8.161	18.720	4.718
Lactobacillaceae	2.740	4.885	2.424	3.665	1.890	3.021	1.246	2.215
Leuconostocaceae	0.023	0.043	0.003	0.010	0.008	0.023	0.012	0.032
Methanobacteriaceae	0.017	0.053	0.012	0.039	0.021	0.046	0.047	0.103
Micrococcaceae	0.003	0.007	0.002	0.007	0.000	0.000	0.001	0.003
Moraxellaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Neisseriaceae	0.000	0.000	0.003	0.010	0.003	0.007	0.000	0.000
Oxalobacteraceae	0.001	0.003	0.000	0.000	0.001	0.003	0.000	0.000
Pasteurellaceae	0.091	0.205	0.060	0.108	0.060	0.127	0.019	0.038
Peptococcaceae	0.001	0.003	0.004	0.007	0.021	0.068	0.032	0.098
Peptostreptococcaceae	0.013	0.017	0.095	0.219	0.020	0.033	0.015	0.032
Phyllobacteriaceae	0.000	0.000	0.000	0.000	0.002	0.004	0.002	0.004
Planococcaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Porphyromonadaceae	1.079	0.923	1.416	1.363	0.636	0.706	1.873	1.292
Prevotellaceae	5.400	12.134	3.315	9.746	0.433	1.005	3.342	6.626
Pseudomonadaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Rikenellaceae	1.584	1.752	0.920	0.804	0.731	1.051	1.685	1.326
Ruminococcaceae	19.689	8.136	14.726	11.386	11.002	10.720	13.084	6.129
S24-7	0.092	0.236	0.109	0.215	0.038	0.063	0.673	1.498
Staphylococcaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.001	0.003
Streptococcaceae	3.987	3.900	4.386	5.042	2.234	2.618	3.683	4.432
Succinivibrionaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.001	0.003
Turicibacteraceae	0.061	0.123	0.079	0.181	0.029	0.041	0.012	0.020
Veillonellaceae	2.257	1.066	5.123	4.190	4.523	5.240	4.916	3.933
Verrucomicrobiaceae	0.058	0.132	0.064	0.142	0.066	0.150	0.041	0.089
Victivallaceae	0.006	0.014	0.004	0.009	0.004	0.009	0.007	0.017

Genus	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
[Eubacterium]	0.418	0.863	0.814	1.352	4.158	5.669	2.526	4.891
[Prevotella]	0.810	2.133	0.125	0.397	0.005	0.009	0.100	0.276
[Ruminococcus]	1.322	0.791	0.851	0.603	0.819	0.698	0.900	0.773
Acidaminococcus	0.405	0.671	0.211	0.355	0.143	0.328	0.772	1.535
Actinobacillus	0.005	0.013	0.000	0.000	0.000	0.000	0.000	0.000
Actinomyces	0.037	0.034	0.020	0.015	0.010	0.009	0.009	0.018
Adlercreutzia	0.052	0.096	0.020	0.042	0.044	0.125	0.034	0.098
Aggregatibacter	0.002	0.006	0.001	0.004	0.000	0.000	0.000	0.000
Akkermansia	0.081	0.183	0.099	0.231	0.121	0.303	0.051	0.108
Anaerococcus	0.000	0.000	0.001	0.004	0.001	0.004	0.002	0.008
Anaerofustis	0.003	0.009	0.002	0.006	0.001	0.004	0.000	0.000
Anaerostipes	0.169	0.127	0.054	0.060	0.054	0.050	0.040	0.043
Anaerotruncus	0.004	0.014	0.005	0.010	0.008	0.026	0.029	0.049
Atopobium	0.002	0.006	0.005	0.008	0.002	0.008	0.002	0.006
Bacteroides	39.13	16.422	38.538	16.194	29.537	18.463	34.616	14.670
Bifidobacterium	4.560	6.057	5.711	8.951	10.338	16.508	9.350	12.643
Bilophila	0.156	0.109	0.862	0.727	1.711	1.365	1.948	1.385
Blautia	6.665	2.501	3.932	1.502	4.527	2.741	4.698	2.313
Bulleidia	0.020	0.057	0.021	0.062	0.158	0.499	0.042	0.133
Butyricimonas	0.109	0.126	0.098	0.114	0.076	0.093	0.167	0.215
Campylobacter	0.007	0.019	0.005	0.007	0.010	0.023	0.004	0.012
Cardiobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Catenibacterium	0.070	0.220	0.079	0.243	5.863	18.514	1.234	3.894
cc_115	0.009	0.018	0.016	0.034	0.033	0.059	0.006	0.010
Christensenella	0.002	0.005	0.002	0.005	0.000	0.000	0.004	0.007
Citrobacter	0.015	0.032	0.868	2.702	1.104	2.454	0.940	2.513
Clostridium	0.104	0.118	1.641	4.756	1.721	4.698	0.183	0.311
Clostridium	0.000	0.000	0.000	0.000	0.011	0.034	0.000	0.000
Collinsella	0.618	0.690	0.651	0.484	1.296	2.716	2.044	2.266
Coprobacillus	0.014	0.023	0.036	0.078	0.125	0.253	0.004	0.006
Coprococcus	5.411	3.727	2.976	1.660	4.505	4.272	4.306	2.603
Corynebacterium	0.000	0.000	0.001	0.004	0.001	0.004	0.000	0.000
Dehalobacterium	0.007	0.016	0.000	0.000	0.000	0.000	0.000	0.000
Delftia	0.000	0.000	0.000	0.000	0.001	0.004	0.000	0.000

Desulfovibrio	0.167	0.348	0.347	0.606	0.286	0.590	0.156	0.351
Dialister	0.361	0.449	0.748	1.397	0.717	1.260	0.662	1.138
Dorea	1.145	0.702	1.613	1.358	2.214	2.518	2.514	2.359
Eggerthella	0.092	0.146	0.143	0.252	0.182	0.357	0.323	0.609
Eikenella	0.000	0.000	0.006	0.018	0.005	0.012	0.000	0.000
Enterobacter	0.004	0.011	0.004	0.012	0.000	0.000	0.000	0.000
Enterococcus	0.684	1.346	0.487	1.239	0.482	1.070	1.344	2.910
Epulopiscium	0.004	0.012	0.007	0.013	0.009	0.023	0.008	0.013
Erwinia	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.006
Escherichia	0.076	0.237	0.320	0.330	0.334	0.532	0.239	0.306
Facklamia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Faecalibacterium	4.941	2.837	3.566	4.216	2.246	3.241	1.103	0.732
Finegoldia	0.002	0.006	0.005	0.010	0.003	0.008	0.018	0.029
Fusobacterium	0.065	0.199	2.385	4.921	1.623	3.318	0.848	2.017
Gemella	0.001	0.004	0.002	0.006	0.000	0.000	0.000	0.000
Granulicatella	0.017	0.018	0.005	0.008	0.002	0.007	0.001	0.004
Haemophilus	0.150	0.374	0.095	0.190	0.093	0.204	0.032	0.069
Helicobacter	0.003	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.020	0.031	0.021	0.030	0.023	0.041	0.020	0.024
Klebsiella	0.046	0.144	0.085	0.251	0.082	0.251	0.068	0.134
Lachnobacterium	0.140	0.262	0.005	0.016	0.014	0.030	0.021	0.044
Lachnospira	1.852	1.107	0.949	0.984	0.512	0.607	0.382	0.513
Lactobacillus	3.273	5.698	2.979	4.539	2.369	3.504	1.467	2.606
Lactococcus	0.018	0.043	0.012	0.023	0.012	0.021	0.000	0.000
Lautropia	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Leuconostoc	0.014	0.033	0.000	0.000	0.001	0.004	0.003	0.005
Luteolibacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Megamonas	0.059	0.188	0.312	0.658	0.609	1.301	0.204	0.646
Megasphaera	0.083	0.258	1.860	3.900	1.507	3.813	1.690	3.727
Mesorhizobium	0.000	0.000	0.000	0.000	0.004	0.008	0.003	0.005
Methanobrevibacter	0.023	0.073	0.021	0.065	0.042	0.092	0.063	0.139
Mogibacterium	0.000	0.000	0.002	0.007	0.002	0.005	0.000	0.000
Morganella	0.004	0.012	0.094	0.299	0.083	0.254	0.011	0.033
Moryella	0.003	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Odoribacter	0.373	0.278	0.329	0.281	0.172	0.183	0.198	0.183
Oribacterium	0.005	0.011	0.000	0.000	0.000	0.000	0.000	0.000
Oscillospira	0.985	0.686	1.962	1.216	2.382	2.145	3.080	2.463

Other	0.000	0.000	0.001	0.005	0.008	0.025	0.000	0.000
Parabacteroides	1.460	1.279	2.063	2.277	0.977	0.943	2.653	1.922
Paraprevotella	0.376	0.694	0.290	0.564	0.981	2.410	0.083	0.110
Pediococcus	0.002	0.008	0.001	0.004	0.000	0.000	0.000	0.000
Peptococcus	0.000	0.000	0.001	0.004	0.038	0.120	0.044	0.138
Peptoniphilus	0.001	0.004	0.004	0.007	0.018	0.026	0.008	0.020
Peptostreptococcus	0.004	0.012	0.010	0.019	0.018	0.034	0.015	0.036
Phascolarctobacterium	1.214	1.327	2.232	1.921	1.588	1.512	2.261	2.031
Porphyromonas	0.001	0.004	0.000	0.000	0.000	0.000	0.001	0.004
Prevotella	6.480	14.281	4.270	12.273	0.711	1.758	4.384	7.935
Providencia	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
PSB-M-3	0.001	0.004	0.001	0.004	0.000	0.000	0.000	0.000
Pseudoramibacter_Eubacterium	0.001	0.004	0.000	0.000	0.004	0.009	0.005	0.010
Pyramidobacter	0.007	0.023	0.014	0.043	0.011	0.034	0.006	0.021
Roseburia	2.022	2.590	0.636	1.430	0.172	0.351	0.089	0.099
Rothia	0.004	0.009	0.003	0.008	0.000	0.000	0.001	0.004
Ruminococcus	5.521	5.319	2.729	2.912	2.005	1.981	3.234	2.649
Scardovia	0.002	0.005	0.003	0.008	0.001	0.004	0.000	0.000
Selenomonas	0.005	0.013	0.002	0.006	0.005	0.012	0.000	0.000
Serratia	0.008	0.021	0.035	0.080	0.215	0.608	0.017	0.054
Slackia	0.054	0.075	0.035	0.071	0.039	0.065	0.033	0.037
SMB53	0.051	0.089	0.065	0.099	0.060	0.091	0.013	0.022
Staphylococcus	0.000	0.000	0.000	0.000	0.002	0.006	0.001	0.004
Streptococcus	5.282	4.656	5.777	6.769	5.515	9.886	4.743	5.552
Sutterella	1.612	0.698	4.249	2.038	3.520	3.432	2.629	1.191
Tetragenococcus	0.000	0.000	0.002	0.006	0.000	0.000	0.000	0.000
Trabulsiella	0.002	0.006	0.000	0.000	0.004	0.009	0.003	0.006
Turicibacter	0.085	0.172	0.115	0.260	0.102	0.180	0.018	0.031
Veillonella	0.975	1.960	1.452	3.136	1.611	3.998	1.285	3.109

Table A4. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon lactulose treatment, over time.

Phylum	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
Actinobacteria	5.385	4.034	17.046	17.075	18.106	18.620	13.574	15.219
Bacteroidetes	32.846	8.510	28.490	11.340	26.338	8.481	29.369	12.084
Cyanobacteria	0.005	0.011	0.014	0.031	0.001	0.003	0.002	0.004
Euryarchaeota	0.018	0.034	0.002	0.006	0.007	0.016	0.051	0.112
Firmicutes	57.534	8.794	47.434	11.446	46.761	13.440	46.205	16.369
Fusobacteria	0.071	0.216	0.660	2.061	1.599	3.849	0.839	2.153
Lentisphaerae	0.004	0.010	0.003	0.010	0.003	0.007	0.012	0.038
Proteobacteria	3.804	6.999	6.300	5.480	7.156	7.264	9.845	6.653
Synergistetes	0.014	0.033	0.001	0.003	0.006	0.019	0.010	0.028
Tenericutes	0.009	0.025	0.000	0.000	0.001	0.003	0.001	0.003
TM7	0.004	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Verrucomicrobia	0.066	0.154	0.050	0.132	0.022	0.066	0.091	0.156

Order	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
Actinomycetales	0.025	0.029	0.006	0.011	0.008	0.016	0.010	0.025
Bacteroidales	32.925	8.524	28.490	11.340	26.338	8.481	29.371	12.086
Bifidobacteriales	4.528	4.029	13.480	17.163	15.345	17.678	10.049	14.994
Burkholderiales	1.047	0.542	1.539	0.681	1.671	0.652	2.758	2.100
Campylobacterales	0.002	0.006	0.001	0.003	0.000	0.000	0.004	0.007
Clostridiales	41.801	13.263	37.226	14.083	28.996	15.070	33.855	10.972
Coriobacteriales	0.846	0.654	3.560	4.049	2.754	3.710	3.515	4.268
Desulfovibrionales	0.202	0.203	0.211	0.158	0.653	0.640	1.628	1.291
Enterobacteriales	2.491	6.892	4.531	5.201	4.829	6.717	5.444	5.651
Erysipelotrichales	1.588	1.001	5.070	4.976	12.896	18.766	4.233	6.171
Fusobacteriales	0.072	0.216	0.660	2.061	1.599	3.849	0.839	2.153
Gemellales	0.006	0.013	0.001	0.003	0.000	0.000	0.001	0.003
I025	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Lactobacillales	14.217	15.395	5.127	4.340	4.854	4.636	8.111	9.281
Methanobacteriales	0.018	0.034	0.002	0.006	0.007	0.016	0.051	0.112
ML615J-28	0.006	0.019	0.000	0.000	0.001	0.003	0.000	0.000
Neisseriales	0.001	0.003	0.002	0.006	0.000	0.000	0.004	0.013

Pasteurellales	0.065	0.161	0.014	0.031	0.002	0.004	0.004	0.007
RF32	0.002	0.004	0.002	0.006	0.000	0.000	0.000	0.000
RF39	0.003	0.007	0.000	0.000	0.000	0.000	0.001	0.003
Rhizobiales	0.001	0.003	0.000	0.000	0.001	0.003	0.000	0.000
SHA-98	0.001	0.003	0.000	0.000	0.001	0.003	0.001	0.003
Streptophyta	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Synergistales	0.014	0.033	0.001	0.003	0.006	0.019	0.010	0.028
Turicibacterales	0.062	0.135	0.010	0.022	0.014	0.030	0.005	0.011
Verrucomicrobiales	0.066	0.154	0.050	0.132	0.022	0.066	0.091	0.156
Victivallales	0.004	0.010	0.003	0.010	0.003	0.007	0.012	0.038
YS2	0.002	0.006	0.014	0.031	0.001	0.003	0.002	0.004

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.386	0.452	0.230	0.296	0.219	0.297	0.445	0.701
[Mogibacteriaceae]	0.111	0.161	0.051	0.081	0.156	0.300	0.154	0.256
[Odoribacteraceae]	0.273	0.225	0.108	0.071	0.124	0.162	0.249	0.190
[Paraprevotellaceae]	0.496	0.583	0.428	0.838	0.207	0.193	0.182	0.270
[Tissierellaceae]	0.003	0.007	0.003	0.005	0.001	0.003	0.009	0.014
Actinomycetaceae	0.019	0.024	0.006	0.011	0.008	0.017	0.004	0.010
Aerococcaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Alcaligenaceae	1.090	0.575	1.579	0.679	1.726	0.685	2.825	2.118
Anaerolinaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bacteroidaceae	24.876	10.438	22.383	11.771	16.506	10.170	22.343	11.965
Bifidobacteriaceae	4.765	4.288	13.954	17.397	15.803	18.007	10.357	15.221
Burkholderiaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Campylobacteraceae	0.002	0.007	0.000	0.000	0.000	0.000	0.004	0.007
Carnobacteriaceae	0.005	0.010	0.002	0.004	0.003	0.005	0.007	0.012
Christensenellaceae	0.089	0.242	0.020	0.046	0.049	0.121	0.034	0.056
Clostridiaceae	0.997	1.296	0.399	0.471	0.400	0.449	0.377	0.383
Comamonadaceae	0.000	0.000	0.001	0.003	0.001	0.003	0.001	0.003
Coriobacteriaceae	0.885	0.688	3.726	4.262	2.913	3.964	3.658	4.420
Dehalobacteriaceae	0.008	0.012	0.001	0.003	0.002	0.005	0.001	0.003
Desulfovibrionaceae	0.212	0.211	0.219	0.163	0.691	0.694	1.668	1.310
Dethiosulfovibrionaceae	0.015	0.034	0.001	0.003	0.007	0.021	0.011	0.031
Enterobacteriaceae	2.555	7.050	4.614	5.200	5.020	6.944	5.550	5.694
Enterococcaceae	0.145	0.308	2.296	3.322	0.878	2.081	0.755	1.610

Erysipelotrichaceae	1.656	1.042	5.185	5.095	13.058	18.782	4.408	6.544
Eubacteriaceae	0.002	0.004	0.001	0.003	0.004	0.010	0.016	0.036
Flavobacteriaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacteriaceae	0.072	0.218	0.662	2.070	1.603	3.856	0.843	2.162
Gemellaceae	0.006	0.013	0.001	0.003	0.000	0.000	0.001	0.003
Helicobacteraceae	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Lachnospiraceae	18.798	5.537	20.577	8.585	12.635	7.575	15.332	7.445
Lactobacillaceae	3.462	4.721	1.528	2.730	1.695	2.406	3.962	7.306
Leuconostocaceae	0.002	0.004	0.006	0.016	0.004	0.013	0.012	0.017
Methanobacteriaceae	0.019	0.035	0.002	0.007	0.007	0.018	0.055	0.121
Micrococcaceae	0.007	0.014	0.000	0.000	0.000	0.000	0.005	0.016
Neisseriaceae	0.001	0.003	0.002	0.007	0.000	0.000	0.004	0.013
Oxalobacteraceae	0.002	0.007	0.000	0.000	0.001	0.003	0.001	0.003
Pasteurellaceae	0.066	0.165	0.014	0.032	0.002	0.004	0.004	0.007
Peptococcaceae	0.037	0.113	0.002	0.007	0.062	0.190	0.264	0.834
Peptostreptococcaceae	0.017	0.021	0.009	0.016	0.003	0.007	0.014	0.035
Phyllobacteriaceae	0.001	0.003	0.000	0.000	0.001	0.003	0.000	0.000
Porphyromonadaceae	1.400	1.303	0.888	0.852	1.155	0.895	2.262	1.776
Prevotellaceae	4.138	11.677	4.585	11.388	7.512	12.720	2.306	4.420
Propionibacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Rikenellaceae	1.426	1.308	0.631	0.676	0.849	1.268	1.078	1.152
Ruminococcaceae	17.331	7.199	11.772	6.492	10.662	6.958	11.485	6.970
S24-7	1.198	3.762	0.062	0.161	0.503	1.431	1.105	2.888
Streptococcaceae	11.139	13.787	1.379	1.225	2.403	3.047	3.540	3.989
Turicibacteraceae	0.066	0.143	0.011	0.024	0.015	0.032	0.005	0.011
Veillonellaceae	2.200	1.166	2.602	1.941	3.085	3.291	4.555	2.330
Verrucomicrobiaceae	0.069	0.160	0.054	0.144	0.024	0.072	0.095	0.163
Victivallaceae	0.004	0.010	0.003	0.010	0.003	0.007	0.013	0.040

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.679	1.257	1.544	2.982	5.163	6.649	3.316	5.608
[Prevotella]	0.213	0.484	0.473	1.239	0.194	0.292	0.107	0.231
[Ruminococcus]	1.111	0.752	1.596	1.523	0.681	0.753	1.341	1.672
Acidaminococcus	0.412	0.703	0.162	0.227	0.205	0.374	0.954	1.854
Actinobacillus	0.004	0.013	0.003	0.008	0.000	0.000	0.000	0.000
Actinomyces	0.027	0.036	0.007	0.013	0.013	0.029	0.005	0.013

Adlercreutzia	0.029	0.052	0.036	0.066	0.079	0.167	0.033	0.066
Aggregatibacter	0.002	0.006	0.003	0.010	0.000	0.000	0.000	0.000
Akkermansia	0.090	0.216	0.067	0.175	0.029	0.086	0.123	0.207
Anaerococcus	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Anaerofustis	0.003	0.006	0.001	0.005	0.007	0.017	0.000	0.000
Anaerostipes	0.204	0.118	0.124	0.168	0.082	0.130	0.121	0.201
Anaerotruncus	0.004	0.007	0.007	0.017	0.017	0.033	0.016	0.029
Atopobium	0.001	0.005	0.001	0.004	0.002	0.006	0.000	0.000
Bacteroides	34.22	15.44	30.18	15.41	23.38	13.34	28.40	15.20
Bifidobacterium	6.390	5.784	19.00	23.987	20.783	24.435	13.032	19.095
Bilophila	0.118	0.129	0.253	0.221	0.924	0.890	2.104	1.724
Blautia	5.344	2.615	7.394	4.549	4.470	2.751	5.859	4.897
Bulleidia	0.010	0.030	0.035	0.110	0.077	0.163	0.105	0.225
Butyricimonas	0.108	0.163	0.036	0.065	0.030	0.061	0.138	0.207
Campylobacter	0.004	0.013	0.000	0.000	0.000	0.000	0.005	0.009
Catenibacterium	0.080	0.253	0.734	2.321	1.770	3.733	1.035	2.487
cc_115	0.008	0.011	0.003	0.008	0.001	0.004	0.003	0.006
Christensenella	0.001	0.004	0.001	0.004	0.004	0.008	0.008	0.014
Chryseobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Citrobacter	0.001	0.004	0.208	0.642	0.027	0.073	0.934	2.885
Clostridium	0.099	0.192	0.243	0.590	0.238	0.392	0.263	0.375
Clostridium	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Clostridium	0.000	0.000	0.000	0.000	0.002	0.007	0.001	0.004
Collinsella	0.690	0.814	4.405	5.216	3.310	5.020	3.908	5.246
Coprobacillus	0.011	0.024	0.004	0.007	0.011	0.018	0.005	0.009
Coprococcus	4.789	2.741	5.495	3.289	3.455	3.099	2.776	2.232
Dehalobacterium	0.011	0.015	0.001	0.005	0.003	0.007	0.001	0.004
Delftia	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Desulfovibrio	0.154	0.272	0.033	0.086	0.128	0.326	0.068	0.183
Dialister	0.506	0.657	0.286	0.538	0.349	0.564	0.511	0.826
Dorea	1.012	0.650	1.283	0.844	1.344	1.471	2.227	2.194
Eggerthella	0.066	0.130	0.053	0.058	0.039	0.045	0.121	0.196
Eikenella	0.002	0.006	0.003	0.010	0.000	0.000	0.005	0.016
Enterobacter	0.002	0.008	0.000	0.000	0.001	0.004	0.009	0.020
Enterococcus	0.239	0.593	2.321	3.649	0.608	1.161	0.478	0.737
Epulopiscium	0.004	0.013	0.002	0.005	0.002	0.006	0.000	0.000
Escherichia	0.089	0.267	0.138	0.165	0.212	0.346	0.146	0.124
Faecalibacterium	4.464	2.806	2.645	2.548	1.973	1.418	1.495	1.517

Finegoldia	0.000	0.000	0.003	0.006	0.002	0.007	0.009	0.015
Flavobacterium	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacterium	0.091	0.267	0.807	2.516	2.216	4.764	0.953	2.400
Gemella	0.000	0.000	0.002	0.005	0.000	0.000	0.000	0.000
Granulicatella	0.005	0.012	0.003	0.006	0.004	0.006	0.007	0.014
Haemophilus	0.113	0.303	0.015	0.037	0.002	0.005	0.005	0.009
Helicobacter	0.000	0.000	0.001	0.005	0.000	0.000	0.000	0.000
Holdemania	0.019	0.046	0.019	0.022	0.016	0.022	0.034	0.050
Klebsiella	0.055	0.174	0.052	0.111	0.075	0.157	0.212	0.426
Lachnobacterium	0.077	0.128	0.059	0.095	0.012	0.033	0.024	0.046
Lachnospira	1.538	0.959	0.353	0.386	0.381	0.599	0.387	0.461
Lactobacillus	4.002	5.422	1.840	3.234	1.984	2.780	5.020	9.686
Lactococcus	0.009	0.028	0.009	0.027	0.001	0.004	0.005	0.014
Leuconostoc	0.003	0.006	0.001	0.005	0.007	0.023	0.010	0.016
Megamonas	0.068	0.209	0.337	1.065	0.574	1.804	0.213	0.673
Megasphaera	0.074	0.233	0.314	0.978	0.321	0.927	0.494	1.295
Mesorhizobium	0.001	0.005	0.000	0.000	0.002	0.005	0.000	0.000
Methanobrevibacter	0.024	0.046	0.003	0.008	0.009	0.021	0.070	0.152
Mogibacterium	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Morganella	0.000	0.000	0.032	0.101	0.000	0.000	0.050	0.155
Odoribacter	0.255	0.222	0.111	0.074	0.155	0.226	0.180	0.109
Oribacterium	0.001	0.004	0.001	0.005	0.001	0.004	0.000	0.000
Oscillospira	0.925	0.816	0.902	0.794	1.823	2.772	2.638	2.563
Other	0.001	0.004	0.000	0.000	0.000	0.000	0.001	0.004
Parabacteroides	1.802	1.690	1.222	1.317	1.579	1.319	2.904	2.353
Paraprevotella	0.400	0.611	0.148	0.233	0.093	0.143	0.115	0.152
Parvimonas	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Pediococcus	0.000	0.000	0.000	0.000	0.001	0.004	0.000	0.000
Peptococcus	0.046	0.145	0.000	0.000	0.073	0.230	0.350	1.106
Peptoniphilus	0.001	0.005	0.000	0.000	0.000	0.000	0.003	0.005
Peptostreptococcus	0.005	0.016	0.006	0.016	0.002	0.007	0.011	0.036
Phascolarctobacterium	1.029	1.049	1.572	1.448	2.190	1.139	2.972	1.429
Prevotella	4.806	13.383	5.794	13.597	10.361	16.396	2.830	5.322
Proteus	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.009
PSB-M-3	0.000	0.000	0.000	0.000	0.001	0.004	0.000	0.000
Pseudoramibacter_Eubacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.045
Pyramidobacter	0.019	0.046	0.001	0.004	0.008	0.025	0.013	0.038
Ralstonia	0.003	0.009	0.000	0.000	0.000	0.000	0.001	0.004

Roseburia	1.971	2.460	0.205	0.243	0.114	0.132	0.095	0.126
Rothia	0.010	0.018	0.000	0.000	0.000	0.000	0.007	0.022
Ruminococcus	5.070	5.394	2.706	2.461	2.352	3.101	2.000	1.924
Scardovia	0.002	0.008	0.001	0.004	0.004	0.008	0.006	0.017
Selenomonas	0.004	0.013	0.000	0.000	0.000	0.000	0.000	0.000
Serratia	0.001	0.005	0.019	0.060	0.006	0.015	0.048	0.118
Slackia	0.072	0.094	0.049	0.085	0.071	0.148	0.071	0.094
SMB53	0.077	0.128	0.012	0.019	0.014	0.042	0.007	0.009
Streptococcus	13.725	15.471	1.782	1.447	3.209	4.275	4.512	5.064
Sutterella	1.484	0.844	2.097	0.894	2.428	1.115	3.553	2.717
Tetragenococcus	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Trabulsiella	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Turicibacter	0.091	0.198	0.014	0.030	0.022	0.048	0.007	0.015
Veillonella	0.904	1.923	0.715	1.400	0.270	0.615	0.500	0.607

Table A5. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon lactulose + rifaximin treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	3.543	2.553	3.517	3.034	4.627	8.084	5.936	12.394
Bacteroidetes	35.960	9.493	29.348	8.662	24.193	14.894	10.069	12.907
Cyanobacteria	0.003	0.007	0.009	0.019	0.000	0.000	0.000	0.000
Euryarchaeota	0.010	0.022	0.016	0.035	0.020	0.042	0.104	0.328
Firmicutes	58.915	9.264	49.089	13.323	53.334	8.056	67.743	12.884
Fusobacteria	0.137	0.293	2.372	7.461	3.322	10.496	0.623	1.970
Lentisphaerae	0.007	0.016	0.001	0.003	0.009	0.015	0.005	0.013
Proteobacteria	1.384	0.891	15.605	17.349	14.392	14.318	15.447	12.697
Synergistetes	0.002	0.006	0.003	0.010	0.005	0.016	0.019	0.061
Tenericutes	0.011	0.024	0.005	0.013	0.000	0.000	0.000	0.000
TM7	0.001	0.003	0.000	0.000	0.001	0.003	0.001	0.003
Verrucomicrobia	0.027	0.073	0.035	0.101	0.098	0.302	0.053	0.169

Order	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
Actinomycetales	0.027	0.032	0.023	0.029	0.006	0.010	0.008	0.010
Aeromonadales	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Bacillales	0.000	0.000	0.000	0.000	0.001	0.003	0.001	0.003
Bacteroidales	35.961	9.493	29.349	8.660	24.193	14.895	10.068	12.908
Bifidobacteriales	2.576	2.204	2.896	3.063	3.413	6.371	5.642	12.351
Burkholderiales	0.765	0.471	1.660	1.553	1.949	1.602	3.725	2.723
Campylobacterales	0.002	0.004	0.001	0.003	0.008	0.015	0.023	0.047
Clostridiales	39.225	12.383	26.775	10.279	31.892	16.949	18.961	9.775
Coriobacteriales	0.940	0.595	0.597	0.444	1.208	1.779	0.286	0.189
Desulfovibrionales	0.177	0.194	0.576	0.465	0.967	0.775	1.495	0.500
Enterobacteriales	0.408	0.722	13.325	16.767	11.448	14.170	10.196	12.871
Erysipelotrichales	1.884	1.533	8.345	8.312	18.600	19.868	43.828	10.653
Fusobacteriales	0.137	0.293	2.372	7.461	3.322	10.496	0.623	1.970
Gemellales	0.004	0.010	0.002	0.006	0.000	0.000	0.001	0.003
Lactobacillales	17.764	13.203	13.949	13.671	2.823	4.826	4.939	5.455
Methanobacteriales	0.010	0.022	0.016	0.035	0.020	0.042	0.104	0.328
ML615J-28	0.007	0.022	0.001	0.003	0.000	0.000	0.000	0.000
Pasteurellales	0.022	0.040	0.027	0.058	0.016	0.028	0.002	0.004
Pseudomonadales	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
RF32	0.006	0.019	0.009	0.017	0.003	0.010	0.000	0.000
RF39	0.004	0.013	0.004	0.010	0.000	0.000	0.000	0.000
Rhizobiales	0.002	0.004	0.001	0.003	0.000	0.000	0.001	0.003
SHA-98	0.005	0.013	0.000	0.000	0.000	0.000	0.000	0.000
Sphingobacteriales	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Sphingomonadales	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004
Synergistales	0.002	0.006	0.003	0.010	0.005	0.016	0.019	0.061
Turicibacterales	0.034	0.060	0.022	0.039	0.019	0.047	0.014	0.025
Verrucomicrobiales	0.027	0.073	0.035	0.101	0.098	0.302	0.053	0.169
Victivallales	0.007	0.016	0.001	0.003	0.009	0.015	0.005	0.013
YS2	0.002	0.006	0.009	0.019	0.000	0.000	0.000	0.000

Family	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
[Barnesiellaceae]	0.271	0.281	0.211	0.217	0.369	0.383	0.053	0.070
[Mogibacteriaceae]	0.120	0.117	0.093	0.145	0.146	0.195	0.022	0.032

[Odoribacteraceae]	0.301	0.291	0.298	0.254	0.220	0.231	0.026	0.036
[Paraprevotellaceae]	0.619	0.553	0.737	1.186	0.346	0.462	0.071	0.166
[Tissierellaceae]	0.003	0.005	0.004	0.010	0.005	0.010	0.113	0.341
Actinomycetaceae	0.018	0.022	0.014	0.015	0.003	0.007	0.005	0.007
Aerococcaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Alcaligenaceae	0.787	0.477	1.681	1.560	2.022	1.661	3.762	2.760
Bacillaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Bacteroidaceae	24.461	15.092	21.171	8.326	18.445	12.977	8.832	11.287
Bifidobacteriaceae	2.701	2.287	2.946	3.079	3.631	6.911	5.677	12.430
Burkholderiaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Campylobacteraceae	0.002	0.004	0.001	0.003	0.008	0.015	0.023	0.047
Carnobacteriaceae	0.007	0.012	0.003	0.007	0.000	0.000	0.002	0.004
Christensenellaceae	0.085	0.180	0.066	0.169	0.027	0.044	0.029	0.060
Clostridiaceae	0.541	0.470	0.803	1.386	0.403	0.429	0.305	0.595
Comamonadaceae	0.000	0.000	0.000	0.000	0.002	0.004	0.000	0.000
Coriobacteriaceae	0.990	0.637	0.618	0.468	1.281	1.929	0.289	0.190
Corynebacteriaceae	0.003	0.007	0.000	0.000	0.000	0.000	0.001	0.003
Dehalobacteriaceae	0.002	0.004	0.003	0.007	0.000	0.000	0.000	0.000
Desulfovibrionaceae	0.187	0.207	0.590	0.473	0.999	0.806	1.507	0.504
Dethiosulfovibrionaceae	0.002	0.007	0.003	0.010	0.005	0.017	0.019	0.061
Enterobacteriaceae	0.420	0.741	13.642	17.004	11.671	14.251	10.262	12.922
Enterococcaceae	0.030	0.056	0.473	1.182	0.387	0.747	1.474	3.435
Erysipelotrichaceae	1.986	1.654	8.509	8.407	18.793	19.947	44.193	10.813
Eubacteriaceae	0.002	0.007	0.001	0.003	0.001	0.003	0.002	0.004
Flavobacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacteriaceae	0.138	0.297	2.388	7.511	3.337	10.546	0.626	1.981
Gemellaceae	0.004	0.010	0.002	0.006	0.000	0.000	0.001	0.003
Helicobacteraceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	17.772	5.108	9.167	3.891	14.067	8.298	6.505	2.665
Lactobacillaceae	5.132	5.330	3.266	4.733	1.360	3.296	2.484	4.163
Leptotrichiaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leuconostocaceae	0.006	0.020	0.013	0.027	0.008	0.017	0.010	0.029
Methanobacteriaceae	0.010	0.023	0.017	0.036	0.021	0.044	0.104	0.330
Microbacteriaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Micrococcaceae	0.007	0.012	0.009	0.026	0.002	0.004	0.002	0.004
Moraxellaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Neisseriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Nocardioidaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Oxalobacteraceae	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Pasteurellaceae	0.023	0.041	0.028	0.058	0.016	0.029	0.002	0.004
Peptococcaceae	0.037	0.110	0.040	0.122	0.200	0.628	0.000	0.000
Peptostreptococcaceae	0.019	0.026	0.111	0.316	0.011	0.021	0.083	0.259
Phyllobacteriaceae	0.002	0.005	0.001	0.003	0.000	0.000	0.001	0.003
Planococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Porphyromonadaceae	1.503	1.180	1.282	1.328	1.692	2.224	0.990	2.933
Prevotellaceae	7.315	14.486	5.278	10.025	2.992	8.371	0.027	0.051
Pseudomonadaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Rikenellaceae	1.383	1.738	1.004	1.191	0.934	0.940	0.112	0.134
Ruminococcaceae	15.692	7.355	12.427	7.188	13.248	8.888	10.729	10.081
S24-7	1.372	4.087	0.128	0.268	0.013	0.040	0.001	0.003
Sphingobacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Sphingomonadaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004
Staphylococcaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Streptococcaceae	13.197	11.147	10.476	11.826	1.113	1.762	0.992	1.272
Succinivibrionaceae	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Synergistaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacteraceae	0.037	0.065	0.023	0.041	0.020	0.050	0.014	0.026
Veillonellaceae	2.774	1.637	2.432	1.456	2.085	1.849	0.582	0.482
Verrucomicrobiaceae	0.028	0.075	0.036	0.103	0.103	0.317	0.054	0.170
Victivallaceae	0.007	0.017	0.001	0.003	0.010	0.016	0.005	0.013

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	1.512	1.841	2.757	2.585	4.049	5.858	7.996	8.866
[Prevotella]	0.326	0.586	0.539	1.318	0.196	0.478	0.000	0.000
[Ruminococcus]	0.892	0.491	1.067	1.196	2.777	3.410	1.753	2.984
Acidaminococcus	0.098	0.250	0.166	0.383	0.734	1.360	0.014	0.036
Actinobacillus	0.000	0.000	0.003	0.011	0.000	0.000	0.000	0.000
Actinomyces	0.021	0.026	0.025	0.032	0.005	0.010	0.009	0.013
Adlercreutzia	0.032	0.066	0.028	0.055	0.048	0.079	0.037	0.097
Aggregatibacter	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Akkermansia	0.034	0.087	0.043	0.119	0.142	0.434	0.089	0.281
Anaerococcus	0.002	0.005	0.002	0.008	0.005	0.013	0.007	0.024
Anaerofustis	0.003	0.009	0.002	0.005	0.000	0.000	0.003	0.010

Anaerostipes	0.197	0.159	0.075	0.108	0.096	0.099	0.027	0.038
Anaerotruncus	0.007	0.014	0.006	0.010	0.013	0.019	0.002	0.005
Atopobium	0.001	0.004	0.003	0.011	0.000	0.000	0.001	0.005
Bacillus	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Bacteroides	31.40	18.79	33.23	14.26	30.16	18.818	17.700	22.120
Bifidobacterium	3.543	3.156	4.908	5.496	6.103	9.221	11.478	20.580
Bilophila	0.142	0.122	0.774	0.636	1.762	1.581	3.055	1.681
Blautia	5.669	2.814	3.772	2.076	5.254	4.099	1.701	1.264
Bulleidia	0.030	0.075	0.018	0.057	0.032	0.102	0.308	0.624
Burkholderia	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Butyricimonas	0.153	0.253	0.144	0.168	0.130	0.235	0.019	0.035
Campylobacter	0.002	0.005	0.002	0.006	0.029	0.066	0.068	0.169
Catenibacterium	0.158	0.333	0.122	0.382	0.091	0.289	10.428	21.961
cc_115	0.040	0.107	0.003	0.008	0.006	0.010	0.029	0.061
Christensenella	0.003	0.006	0.005	0.016	0.000	0.000	0.006	0.014
Citrobacter	0.015	0.026	3.087	9.740	5.398	16.98	6.189	15.91
Clostridium	0.030	0.032	0.837	2.339	0.168	0.233	0.487	1.314
Clostridium	0.000	0.000	0.000	0.000	0.015	0.028	0.009	0.024
Collinsella	0.720	0.696	0.462	0.475	1.384	2.356	0.349	0.390
Coprobacillus	0.013	0.026	0.058	0.126	0.061	0.177	0.095	0.167
Coprococcus	4.274	2.759	1.942	1.418	3.916	3.127	0.737	0.558
Corynebacterium	0.004	0.008	0.000	0.000	0.000	0.000	0.003	0.010
Cryocola	0.000	0.000	0.000	0.000	0.003	0.008	0.000	0.000
Dehalobacterium	0.003	0.006	0.004	0.010	0.000	0.000	0.000	0.000
Desulfovibrio	0.094	0.221	0.062	0.175	0.192	0.406	0.390	0.866
Dialister	0.795	1.113	0.951	1.231	0.590	0.851	0.348	0.719
Dorea	0.845	0.628	0.701	0.631	3.590	4.348	2.723	5.326
Eggerthella	0.006	0.012	0.056	0.156	0.017	0.031	0.020	0.046
Enterobacter	0.005	0.011	0.002	0.008	0.004	0.009	0.002	0.008
Enterococcus	0.034	0.078	0.366	0.681	1.241	2.931	3.842	8.386
Epulopiscium	0.001	0.004	0.006	0.012	0.007	0.018	0.004	0.011
Erwinia	0.000	0.000	0.002	0.006	0.003	0.008	0.009	0.018
Escherichia	0.003	0.006	0.674	1.138	0.536	1.038	0.319	0.497
Faecalibacterium	4.100	2.850	2.841	1.358	2.282	1.684	0.553	0.861
Finegoldia	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Fusobacterium	0.163	0.350	2.864	8.987	4.422	13.96	0.926	2.928
Gemella	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Granulicatella	0.008	0.014	0.006	0.013	0.000	0.000	0.003	0.006

Haemophilus	0.025	0.047	0.071	0.183	0.053	0.128	0.005	0.012
Holdemania	0.011	0.016	0.061	0.178	0.059	0.121	0.057	0.066
Klebsiella	0.090	0.192	0.097	0.298	0.095	0.275	0.082	0.142
Lachnobacterium	0.072	0.131	0.015	0.029	0.068	0.122	0.062	0.163
Lachnospira	1.612	1.191	0.478	0.374	0.782	1.175	0.428	0.550
Lactobacillus	5.888	6.035	3.853	5.406	1.829	4.278	3.169	4.878
Lactococcus	0.019	0.040	0.040	0.115	0.023	0.072	0.067	0.120
Leuconostoc	0.009	0.028	0.007	0.016	0.009	0.027	0.002	0.007
Megamonas	0.124	0.265	0.034	0.108	0.009	0.030	0.009	0.028
Megasphaera	0.199	0.414	0.112	0.327	0.016	0.047	0.013	0.038
Mesorhizobium	0.003	0.006	0.002	0.006	0.000	0.000	0.002	0.008
Methanobrevibacter	0.013	0.027	0.020	0.042	0.028	0.059	0.173	0.546
Mogibacterium	0.001	0.004	0.002	0.006	0.000	0.000	0.003	0.009
Morganella	0.000	0.000	0.014	0.045	0.064	0.143	0.742	2.146
Novosphingobium	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.010
Odoribacter	0.242	0.243	0.293	0.302	0.210	0.201	0.042	0.063
Oscillospira	0.977	0.630	2.429	2.484	3.301	2.721	5.942	4.643
Other	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
p-75-a5	0.003	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Parabacteroides	1.966	1.596	1.716	1.896	2.292	2.859	1.497	4.325
Paraprevotella	0.395	0.510	0.330	0.494	0.259	0.447	0.108	0.249
Parvimonas	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Pediococcus	0.004	0.010	0.002	0.006	0.001	0.004	0.001	0.005
Pedobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Peptococcus	0.045	0.142	0.049	0.150	0.253	0.801	0.000	0.000
Peptoniphilus	0.000	0.000	0.002	0.005	0.002	0.005	0.325	1.026
Peptostreptococcus	0.010	0.021	0.012	0.028	0.004	0.013	0.121	0.384
Phascolarctobacterium	1.568	1.446	1.710	2.292	1.312	1.633	0.799	1.434
Porphyromonas	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Prevotella	8.381	16.467	6.324	11.618	3.893	10.895	0.032	0.060
Proteus	0.000	0.000	0.007	0.015	0.009	0.017	0.001	0.004
PSB-M-3	0.002	0.008	0.001	0.004	0.000	0.000	0.006	0.019
Pseudomonas	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.008
Pseudoramibacter_Eubacterium	0.000	0.000	0.000	0.000	0.005	0.016	0.002	0.008
Pyramidobacter	0.002	0.008	0.004	0.011	0.007	0.023	0.032	0.101
Roseburia	1.065	1.665	0.324	0.556	0.155	0.187	0.030	0.039
Rothia	0.009	0.014	0.013	0.031	0.003	0.006	0.002	0.005
Ruminococcus	4.128	5.422	2.494	3.061	3.682	4.200	1.913	2.842

Scardovia	0.002	0.005	0.001	0.004	0.000	0.000	0.001	0.004
Selenomonas	0.001	0.004	0.005	0.011	0.000	0.000	0.000	0.000
Serratia	0.001	0.004	0.032	0.089	0.045	0.118	1.637	4.898
Slackia	0.066	0.066	0.041	0.057	0.046	0.083	0.018	0.038
SMB53	0.060	0.092	0.017	0.029	0.045	0.115	0.020	0.046
Sphingomonas	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.008
Staphylococcus	0.000	0.000	0.000	0.000	0.002	0.005	0.000	0.000
Streptococcus	15.998	13.280	13.230	13.193	1.830	2.369	1.443	1.483
Sutterella	0.964	0.567	2.691	2.868	3.594	3.245	9.319	9.396
Trabulsiella	0.000	0.000	0.002	0.006	0.013	0.040	0.014	0.025
Turicibacter	0.048	0.086	0.039	0.072	0.048	0.132	0.044	0.091
Veillonella	0.605	0.780	0.805	1.648	0.490	1.003	0.086	0.097
WAL_1855D	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000

Table A6. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon rifaximin treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	3.841	2.0358	4.061	2.194	5.214	8.320	7.346	11.129
Bacteroidetes	28.270	13.964	29.423	13.302	30.533	9.574	22.972	11.063
Cyanobacteria	0.170	0.371	0.011	0.025	0.003	0.010	0.001	0.003
Euryarchaeota	0.000	0.000	0.029	0.065	0.016	0.051	0.000	0.000
Firmicutes	60.962	13.577	56.297	11.252	47.851	11.959	40.278	9.138
Fusobacteria	0.826	2.339	3.406	10.720	2.021	5.288	4.151	10.169
Lentisphaerae	0.026	0.083	0.005	0.009	0.005	0.011	0.007	0.013
Proteobacteria	5.984	1.269	6.489	6.230	14.243	12.079	25.226	17.077
Synergistetes	0.013	0.042	0.009	0.019	0.006	0.019	0.000	0.000
Tenericutes	0.052	0.126	0.006	0.013	0.000	0.000	0.002	0.004
TM7	0.013	0.041	0.001	0.003	0.000	0.000	0.001	0.003
Verrucomicrobia	0.314	0.530	0.262	0.563	0.108	0.293	0.015	0.027

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinomycetales	0.025	0.023	0.025	0.026	0.042	0.042	0.014	0.017

Aeromonadales	0.002	0.006	0.001	0.003	0.001	0.003	0.000	0.000
Bacillales	0.000	0.000	0.003	0.007	0.002	0.006	0.000	0.000
Bacteroidales	37.133	10.740	29.426	13.304	30.535	9.574	22.973	11.062
Bifidobacteriales	2.840	3.106	3.185	2.079	4.615	8.463	6.647	10.657
Burkholderiales	0.955	0.392	1.790	1.441	2.802	1.869	2.414	2.227
Campylobacterales	0.005	0.013	0.003	0.007	0.013	0.021	0.004	0.013
Cardiobacteriales	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Clostridiales	39.563	11.209	36.302	15.786	37.649	12.612	30.142	11.038
Coriobacteriales	0.628	0.405	0.851	0.463	0.557	0.305	0.685	0.604
Desulfovibrionales	0.246	0.231	0.925	0.785	2.769	1.574	5.354	3.092
Enterobacteriales	3.295	9.391	3.740	6.168	8.616	12.745	17.433	15.577
Erysipelotrichales	1.461	1.186	4.828	4.334	3.482	4.843	3.382	3.717
Fusobacteriales	0.064	0.180	3.406	10.720	2.021	5.289	4.151	10.169
Gemellales	0.011	0.021	0.002	0.004	0.003	0.005	0.003	0.007
Lactobacillales	13.576	13.137	15.102	16.834	6.646	9.241	6.693	8.058
Methanobacteriales	0.000	0.000	0.029	0.066	0.016	0.051	0.000	0.000
Pasteurellales	0.088	0.240	0.015	0.031	0.021	0.060	0.019	0.038
Pseudomonadales	0.000	0.000	0.000	0.000	0.003	0.005	0.000	0.000
RF32	0.006	0.013	0.001	0.003	0.010	0.020	0.000	0.000
RF39	0.004	0.010	0.006	0.013	0.000	0.000	0.002	0.004
Rhizobiales	0.002	0.004	0.007	0.008	0.001	0.003	0.002	0.004
SHA-98	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Streptophyta	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Synergistales	0.001	0.003	0.009	0.019	0.006	0.019	0.000	0.000
Turicibacterales	0.054	0.079	0.065	0.114	0.070	0.142	0.060	0.106
Verrucomicrobiales	0.024	0.041	0.262	0.563	0.108	0.293	0.015	0.027
Victivallales	0.002	0.006	0.005	0.009	0.005	0.011	0.007	0.013
YS2	0.013	0.029	0.010	0.026	0.003	0.010	0.001	0.003

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.357	0.388	0.409	0.516	0.396	0.488	0.290	0.476
[Mogibacteriaceae]	0.058	0.050	0.109	0.108	0.093	0.171	0.041	0.052
[Odoribacteraceae]	0.295	0.226	0.330	0.237	0.300	0.252	0.161	0.098
[Paraprevotellaceae]	0.775	1.848	0.914	1.422	0.712	1.128	0.045	0.098
[Tissierellaceae]	0.001	0.003	0.001	0.003	0.011	0.029	0.007	0.016
Actinomycetaceae	0.016	0.023	0.020	0.018	0.032	0.033	0.013	0.018

Aerococcaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Alcaligenaceae	0.990	0.408	1.863	1.555	2.875	1.970	2.465	2.275
Bacteroidaceae	27.693	12.747	20.738	11.382	23.854	10.607	19.027	8.758
Bifidobacteriaceae	2.964	3.227	3.298	2.142	4.700	8.569	6.808	10.990
Campylobacteraceae	0.001	0.003	0.002	0.004	0.010	0.020	0.004	0.013
Cardiobacteriaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Carnobacteriaceae	0.016	0.020	0.009	0.013	0.007	0.007	0.009	0.014
Christensenellaceae	0.025	0.043	0.058	0.106	0.075	0.204	0.016	0.026
Clostridiaceae	0.800	0.811	0.604	0.693	1.996	4.875	1.089	2.093
Comamonadaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Coriobacteriaceae	0.660	0.431	0.885	0.483	0.573	0.316	0.705	0.625
Corynebacteriaceae	0.002	0.004	0.001	0.003	0.001	0.003	0.000	0.000
Dehalobacteriaceae	0.000	0.000	0.001	0.003	0.001	0.003	0.000	0.000
Desulfovibrionaceae	0.260	0.252	0.964	0.822	2.844	1.624	5.451	3.110
Dethiosulfovibrionaceae	0.001	0.003	0.009	0.020	0.006	0.020	0.000	0.000
Enterobacteriaceae	3.355	9.544	3.821	6.192	8.772	12.915	17.728	15.709
Enterococcaceae	0.330	0.763	0.258	0.667	0.419	0.690	1.063	2.341
Erysipelotrichaceae	1.526	1.258	4.950	4.396	3.548	4.910	3.446	3.776
Eubacteriaceae	0.001	0.003	0.000	0.000	0.001	0.003	0.003	0.007
Fusobacteriaceae	0.064	0.182	3.443	10.838	2.045	5.355	4.185	10.236
Gemellaceae	0.011	0.021	0.002	0.004	0.003	0.005	0.003	0.007
Helicobacteraceae	0.004	0.013	0.001	0.003	0.003	0.010	0.000	0.000
Lachnospiraceae	18.092	5.049	13.739	5.529	12.185	4.047	10.266	4.862
Lactobacillaceae	3.582	4.908	3.511	4.922	3.007	6.293	0.814	1.295
Leuconostocaceae	0.005	0.013	0.004	0.010	0.032	0.077	0.004	0.013
Methanobacteriaceae	0.000	0.000	0.030	0.067	0.017	0.052	0.000	0.000
Micrococcaceae	0.008	0.011	0.005	0.010	0.009	0.017	0.001	0.003
Moraxellaceae	0.000	0.000	0.000	0.000	0.003	0.005	0.000	0.000
Nocardioidaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Oxalobacteraceae	0.001	0.003	0.003	0.007	0.003	0.007	0.002	0.004
Pasteurellaceae	0.090	0.244	0.015	0.031	0.021	0.061	0.020	0.039
Peptococcaceae	0.002	0.007	0.000	0.000	0.004	0.014	0.003	0.007
Peptostreptococcaceae	0.013	0.015	0.020	0.029	0.011	0.017	0.010	0.033
Phyllobacteriaceae	0.002	0.005	0.007	0.009	0.001	0.003	0.002	0.004
Porphyromonadaceae	1.100	0.891	1.439	1.278	1.045	0.885	1.463	1.829
Prevotellaceae	5.982	12.495	4.746	10.366	3.376	7.310	0.028	0.063
Rikenellaceae	0.925	0.879	1.712	1.357	1.468	1.702	1.844	3.546
Ruminococcaceae	15.898	7.378	17.815	10.973	20.260	11.406	15.699	11.460

S24-7	1.412	3.691	0.133	0.236	0.068	0.135	0.611	1.037
Staphylococcaceae	0.000	0.000	0.003	0.007	0.002	0.007	0.000	0.000
Streptococcaceae	10.101	10.662	11.645	14.804	3.271	3.787	4.899	7.040
Succinivibrionaceae	0.002	0.006	0.001	0.003	0.001	0.003	0.000	0.000
Synergistaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacteraceae	0.058	0.084	0.069	0.122	0.073	0.146	0.062	0.111
Veillonellaceae	2.493	1.339	2.136	1.553	1.744	1.146	1.689	1.130
Verrucomicrobiaceae	0.025	0.043	0.270	0.579	0.110	0.300	0.015	0.028
Victivallaceae	0.002	0.007	0.005	0.009	0.005	0.012	0.007	0.014

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.786	1.462	1.955	2.473	1.019	1.914	1.894	3.701
[Prevotella]	0.803	2.163	0.713	1.714	0.594	1.324	0.030	0.096
[Ruminococcus]	1.116	0.898	1.176	0.985	1.058	1.034	0.868	0.892
Acidaminococcus	0.170	0.306	0.465	1.040	0.083	0.140	0.107	0.283
Acinetobacter	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Actinobacillus	0.010	0.027	0.000	0.000	0.002	0.007	0.000	0.000
Actinomyces	0.026	0.045	0.026	0.022	0.045	0.041	0.023	0.041
Adlercreutzia	0.044	0.067	0.042	0.071	0.065	0.104	0.017	0.022
Aeromicrobium	0.000	0.000	0.000	0.000	0.001	0.005	0.000	0.000
Aggregatibacter	0.008	0.027	0.000	0.000	0.000	0.000	0.000	0.000
Akkermansia	0.036	0.064	0.386	0.831	0.157	0.429	0.020	0.037
Alistipes	0.001	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Anaerococcus	0.000	0.000	0.000	0.000	0.006	0.018	0.000	0.000
Anaerofustis	0.001	0.004	0.000	0.000	0.000	0.000	0.003	0.010
Anaerostipes	0.114	0.090	0.122	0.101	0.080	0.094	0.025	0.030
Anaerotruncus	0.003	0.006	0.010	0.015	0.004	0.010	0.003	0.011
Atopobium	0.004	0.008	0.003	0.010	0.005	0.009	0.004	0.009
Bacteroides	38.40	17.79	29.04	17.04	34.58	16.386	28.178	10.973
Bifidobacterium	4.152	4.175	4.693	3.170	6.911	12.093	9.586	13.458
Bilophila	0.153	0.103	1.157	1.005	3.629	2.220	8.357	5.118
Blautia	5.351	2.304	4.376	2.722	4.234	2.042	2.689	2.103
Bulleidia	0.020	0.064	0.036	0.114	0.064	0.190	0.102	0.297
Butyricimonas	0.110	0.139	0.088	0.102	0.068	0.080	0.053	0.068
Campylobacter	0.002	0.007	0.003	0.007	0.019	0.044	0.011	0.034
Cardiobacterium	0.002	0.007	0.000	0.000	0.000	0.000	0.000	0.000

Catenibacterium	0.055	0.172	0.139	0.433	0.150	0.475	0.000	0.000
cc_115	0.047	0.108	0.107	0.240	0.020	0.034	0.007	0.016
Christensenella	0.001	0.004	0.002	0.005	0.003	0.010	0.002	0.005
Citrobacter	0.019	0.032	0.014	0.032	2.696	7.032	2.657	6.121
Clostridium	0.098	0.108	0.110	0.158	1.975	5.718	0.977	2.892
Clostridium	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Collinsella	0.494	0.424	0.678	0.596	0.404	0.342	0.496	0.802
Coprobacillus	0.014	0.019	0.040	0.063	0.056	0.105	0.024	0.031
Coprococcus	4.557	3.677	3.738	2.400	2.240	1.652	1.963	2.591
Corynebacterium	0.003	0.006	0.002	0.005	0.002	0.005	0.000	0.000
Dehalobacterium	0.000	0.000	0.001	0.004	0.002	0.005	0.000	0.000
Delftia	0.000	0.000	0.000	0.000	0.001	0.004	0.000	0.000
Desulfovibrio	0.183	0.351	0.172	0.538	0.278	0.563	0.231	0.395
Dialister	0.359	0.755	0.883	1.192	0.616	1.018	0.742	2.079
Dorea	0.920	0.647	0.901	0.750	0.739	0.588	1.109	1.389
Eggerthella	0.052	0.081	0.016	0.019	0.036	0.053	0.149	0.377
Enterobacter	0.004	0.011	0.002	0.008	0.004	0.009	0.013	0.037
Enterococcus	0.505	1.153	0.397	1.043	0.692	1.183	1.513	2.926
Epulopiscium	0.008	0.027	0.002	0.005	0.008	0.015	0.002	0.005
Erwinia	0.000	0.000	0.003	0.010	0.007	0.011	0.003	0.006
Escherichia	0.103	0.321	0.136	0.198	0.385	0.925	0.410	0.715
Faecalibacterium	4.252	2.463	3.419	2.653	2.886	1.870	1.851	1.646
Finegoldia	0.000	0.000	0.000	0.000	0.003	0.011	0.006	0.015
Fusobacterium	0.078	0.214	3.981	12.525	2.340	6.027	4.789	11.705
Granulicatella	0.017	0.023	0.009	0.016	0.008	0.009	0.010	0.017
Haemophilus	0.157	0.450	0.022	0.045	0.045	0.132	0.039	0.080
Helicobacter	0.003	0.010	0.002	0.005	0.005	0.015	0.000	0.000
Holdemania	0.007	0.008	0.049	0.084	0.055	0.086	0.086	0.106
Kaistobacter	0.000	0.000	0.000	0.000	0.001	0.005	0.000	0.000
Klebsiella	0.053	0.165	0.106	0.335	0.131	0.406	0.001	0.004
Lachnobacterium	0.084	0.257	0.119	0.208	0.072	0.176	0.006	0.020
Lachnospira	1.510	1.315	0.959	1.309	1.092	1.055	0.766	1.009
Lactobacillus	4.138	5.605	4.305	5.931	3.636	7.577	0.968	1.485
Lactococcus	0.006	0.012	0.036	0.099	0.013	0.033	0.007	0.022
Leuconostoc	0.001	0.004	0.005	0.013	0.012	0.037	0.005	0.015
Megamonas	0.046	0.144	0.013	0.041	0.029	0.091	0.019	0.055
Megasphaera	0.075	0.231	0.071	0.207	0.167	0.505	0.163	0.426
Mesorhizobium	0.003	0.006	0.009	0.010	0.001	0.004	0.003	0.007

Methanobrevibacter	0.000	0.000	0.043	0.097	0.024	0.075	0.000	0.000
Mogibacterium	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Morganella	0.005	0.012	0.000	0.000	0.067	0.212	0.250	0.790
Odoribacter	0.286	0.239	0.380	0.319	0.354	0.321	0.187	0.130
Oribacterium	0.000	0.000	0.000	0.000	0.003	0.006	0.003	0.008
Oscillospira	0.656	0.297	2.348	1.975	6.037	5.236	5.489	5.221
Other	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
p-75-a5	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.044
Parabacteroides	1.487	1.277	1.972	1.828	1.384	1.142	2.002	2.250
Paraprevotella	0.138	0.279	0.460	0.730	0.297	0.579	0.021	0.062
Parvimonas	0.001	0.004	0.002	0.005	0.000	0.000	0.000	0.000
Pediococcus	0.000	0.000	0.001	0.004	0.001	0.004	0.000	0.000
Peptococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Peptoniphilus	0.000	0.000	0.000	0.000	0.001	0.004	0.002	0.005
Peptostreptococcus	0.002	0.008	0.004	0.011	0.002	0.007	0.011	0.033
ph2	0.000	0.000	0.000	0.000	0.002	0.005	0.000	0.000
Phascolarctobacterium	1.770	1.694	1.175	1.618	1.030	1.237	0.713	0.892
Plesiomonas	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.009
Porphyromonas	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Prevotella	7.146	14.467	5.791	12.568	4.132	8.919	0.056	0.138
Proteus	0.001	0.004	0.005	0.015	0.001	0.004	0.000	0.000
PSB-M-3	0.000	0.000	0.001	0.004	0.002	0.007	0.001	0.004
Pseudoramibacter_Eubacterium	0.000	0.000	0.000	0.000	0.001	0.004	0.001	0.004
Pyramidobacter	0.000	0.000	0.012	0.028	0.009	0.028	0.000	0.000
Ralstonia	0.001	0.005	0.003	0.009	0.004	0.009	0.004	0.009
Roseburia	1.075	1.383	0.351	0.387	0.547	0.878	0.086	0.106
Rothia	0.011	0.014	0.007	0.012	0.012	0.020	0.001	0.004
Ruminococcus	3.164	3.243	3.646	3.432	3.705	3.715	2.625	3.365
Scardovia	0.000	0.000	0.001	0.004	0.000	0.000	0.001	0.004
Selenomonas	0.001	0.004	0.000	0.000	0.001	0.004	0.000	0.000
Serratia	0.010	0.021	0.003	0.010	0.125	0.272	5.889	9.555
Slackia	0.029	0.041	0.055	0.077	0.040	0.089	0.015	0.025
SMB53	0.083	0.140	0.062	0.127	0.068	0.150	0.071	0.115
Staphylococcus	0.000	0.000	0.005	0.011	0.003	0.010	0.000	0.000
Streptococcus	12.560	12.610	16.192	22.119	4.316	4.325	6.851	8.978
Sutterella	1.377	0.759	2.403	2.269	3.838	2.999	3.629	3.061
TG5	0.002	0.007	0.001	0.004	0.000	0.000	0.000	0.000
Trabulsiella	0.000	0.000	0.001	0.004	0.004	0.009	0.003	0.006

Turicibacter	0.076	0.108	0.098	0.171	0.104	0.209	0.108	0.187
Veillonella	0.945	1.976	0.231	0.345	0.441	0.842	0.975	1.779

Table A7. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon VSL#3 treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	4.926	3.794	3.672	2.400	6.609	11.259	14.076	14.264
Bacteroidetes	29.600	11.722	30.377	10.224	23.046	13.549	24.226	7.630
Chloroflexi	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Cyanobacteria	0.011	0.024	0.009	0.029	0.009	0.025	0.001	0.003
Euryarchaeota	0.009	0.025	0.004	0.013	0.009	0.019	0.005	0.016
Firmicutes	62.897	7.610	54.142	12.439	52.691	12.577	49.669	11.586
Fusobacteria	0.010	0.018	1.087	3.413	4.811	15.152	1.296	4.045
Lentisphaerae	0.003	0.005	0.000	0.000	0.000	0.000	0.035	0.101
Proteobacteria	2.467	2.794	10.633	11.836	12.744	12.626	10.608	9.037
Synergistetes	0.003	0.007	0.004	0.010	0.010	0.029	0.024	0.077
Tenericutes	0.014	0.039	0.001	0.003	0.000	0.000	0.000	0.000
TM7	0.003	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Verrucomicrobia	0.057	0.111	0.072	0.188	0.072	0.205	0.061	0.178

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinomycetales	0.021	0.011	0.009	0.013	0.008	0.013	0.013	0.019
Aeromonadales	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Bacillales	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.007
Bacteroidales	29.601	11.722	30.378	10.224	23.046	13.550	24.226	7.630
Bifidobacteriales	4.400	3.944	3.047	2.441	6.138	11.277	11.946	12.365
Burkholderiales	0.840	0.268	2.326	1.528	1.641	1.491	2.659	2.242
Campylobacterales	0.009	0.015	0.002	0.004	0.001	0.003	0.007	0.022
Clostridiales	33.945	11.952	29.440	10.636	22.812	15.440	36.398	10.338
Coriobacteriales	0.505	0.299	0.616	0.431	0.463	0.433	2.117	3.053
Desulfovibrionales	0.154	0.157	0.534	0.342	0.728	0.577	1.755	1.085
Enterobacteriales	1.404	2.887	7.743	11.529	10.358	12.430	6.176	7.632
Erysipelotrichales	0.962	0.854	9.470	16.751	16.533	18.154	2.467	3.006

Fusobacteriales	0.010	0.018	1.087	3.413	4.811	15.152	1.296	4.045
Gemellales	0.005	0.008	0.001	0.003	0.001	0.003	0.001	0.003
Lactobacillales	27.944	13.101	15.223	14.490	13.341	13.047	10.791	8.568
Methanobacteriales	0.009	0.025	0.004	0.013	0.009	0.019	0.005	0.016
ML615J-28	0.013	0.036	0.000	0.000	0.000	0.000	0.000	0.000
Neisseriales	0.000	0.000	0.002	0.006	0.000	0.000	0.001	0.003
Pasteurellales	0.045	0.077	0.019	0.042	0.012	0.024	0.007	0.015
Pseudomonadales	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
RF32	0.015	0.032	0.002	0.004	0.001	0.003	0.001	0.003
RF39	0.001	0.004	0.001	0.003	0.000	0.000	0.000	0.000
Rhizobiales	0.001	0.004	0.001	0.003	0.000	0.000	0.001	0.003
SHA-98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Synergistales	0.003	0.007	0.004	0.010	0.010	0.029	0.024	0.077
Turicibacterales	0.042	0.098	0.009	0.020	0.003	0.010	0.010	0.019
Verrucomicrobiales	0.057	0.111	0.072	0.188	0.072	0.205	0.061	0.178
Victivallales	0.003	0.005	0.000	0.000	0.000	0.000	0.035	0.101
YS2	0.011	0.024	0.009	0.029	0.008	0.026	0.000	0.000

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.238	0.224	0.243	0.298	0.146	0.221	0.353	0.583
[Mogibacteriaceae]	0.049	0.070	0.033	0.047	0.042	0.099	0.202	0.311
[Odoribacteraceae]	0.298	0.259	0.181	0.107	0.107	0.117	0.211	0.149
[Paraprevotellaceae]	0.973	1.897	0.536	0.956	0.397	0.513	0.135	0.219
[Tissierellaceae]	0.000	0.000	0.003	0.007	0.000	0.000	0.005	0.010
Actinomycetaceae	0.016	0.014	0.005	0.007	0.006	0.013	0.009	0.016
Aerococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Alcaligenaceae	0.866	0.282	2.373	1.577	1.686	1.550	2.739	2.304
Bacillaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.001	0.003
Bacteroidaceae	20.549	12.287	21.833	9.090	16.679	11.816	19.203	7.140
Bifidobacteriaceae	4.518	4.034	3.092	2.446	6.227	11.371	12.459	12.928
Campylobacteraceae	0.004	0.008	0.002	0.004	0.001	0.003	0.007	0.023
Carnobacteriaceae	0.006	0.011	0.004	0.007	0.000	0.000	0.002	0.004
Christensenellaceae	0.063	0.162	0.008	0.020	0.006	0.014	0.094	0.173
Clostridiaceae	0.547	0.996	0.760	1.284	0.568	1.256	0.469	0.775
Coriobacteriaceae	0.523	0.311	0.632	0.447	0.473	0.441	2.237	3.273
Corynebacteriaceae	0.000	0.000	0.001	0.003	0.002	0.004	0.002	0.007

Dehalobacteriaceae	0.001	0.004	0.000	0.000	0.000	0.000	0.002	0.007
Desulfovibrionaceae	0.159	0.162	0.548	0.355	0.743	0.592	1.809	1.108
Dethiosulfovibrionaceae	0.003	0.007	0.003	0.010	0.010	0.030	0.025	0.079
Enterobacteriaceae	1.424	2.911	7.820	11.547	10.527	12.597	6.326	7.669
Enterococcaceae	0.318	0.657	0.558	0.914	0.550	0.913	1.075	1.314
Erysipelotrichaceae	0.990	0.881	9.753	17.400	16.618	18.225	2.543	3.088
Eubacteriaceae	0.003	0.005	0.000	0.000	0.001	0.003	0.004	0.007
Fusobacteriaceae	0.010	0.018	1.092	3.429	4.831	15.216	1.317	4.110
Gemellaceae	0.005	0.008	0.001	0.003	0.001	0.003	0.001	0.003
Helicobacteraceae	0.005	0.015	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	15.625	5.544	11.129	5.712	10.075	7.542	17.674	6.243
Lactobacillaceae	5.403	3.287	3.187	3.503	3.011	3.536	2.561	4.086
Leuconostocaceae	0.024	0.041	0.001	0.003	0.007	0.019	0.015	0.028
Methanobacteriaceae	0.009	0.026	0.004	0.013	0.009	0.020	0.005	0.017
Micrococcaceae	0.006	0.011	0.003	0.010	0.000	0.000	0.002	0.004
Neisseriaceae	0.000	0.000	0.002	0.006	0.000	0.000	0.001	0.003
Nocardioidaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Oxalobacteraceae	0.001	0.004	0.002	0.004	0.000	0.000	0.000	0.000
Pasteurellaceae	0.046	0.078	0.020	0.042	0.012	0.025	0.007	0.015
Peptococcaceae	0.000	0.000	0.000	0.000	0.015	0.048	0.000	0.000
Peptostreptococcaceae	0.014	0.022	0.002	0.007	0.015	0.033	0.014	0.028
Phyllobacteriaceae	0.001	0.004	0.001	0.003	0.000	0.000	0.001	0.003
Planococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Porphyromonadaceae	0.803	0.694	1.272	0.959	1.402	1.525	2.099	2.214
Prevotellaceae	6.191	12.442	4.038	9.180	4.003	8.172	0.310	0.954
Pseudomonadaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Rikenellaceae	1.223	1.266	0.709	0.597	0.580	0.756	1.884	1.805
Ruminococcaceae	14.362	7.228	13.069	6.928	8.855	7.412	11.750	7.892
S24-7	0.110	0.291	2.085	4.605	0.122	0.250	0.838	2.608
Staphylococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Streptococcaceae	22.891	11.800	11.668	13.250	10.026	11.578	7.425	7.214
Succinivibrionaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Synergistaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Turicibacteraceae	0.043	0.101	0.009	0.021	0.003	0.010	0.010	0.019
Veillonellaceae	1.617	1.382	3.243	2.422	2.165	2.170	4.067	5.267
Verrucomicrobiaceae	0.059	0.114	0.073	0.192	0.074	0.213	0.063	0.184
Victivallaceae	0.003	0.005	0.000	0.000	0.000	0.000	0.038	0.109

Genus	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
[Eubacterium]	0.374	0.870	1.670	3.669	1.649	2.228	0.317	0.579
[Prevotella]	0.947	2.154	0.521	1.473	0.290	0.695	0.000	0.000
[Ruminococcus]	0.916	0.495	0.711	0.496	1.617	3.667	1.251	0.859
Acidaminococcus	0.251	0.340	0.559	0.977	0.802	1.177	3.534	7.346
Actinobacillus	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Actinomyces	0.020	0.017	0.006	0.011	0.002	0.005	0.009	0.017
Adlercreutzia	0.042	0.072	0.057	0.080	0.033	0.059	0.079	0.116
Akkermansia	0.071	0.134	0.119	0.260	0.113	0.288	0.097	0.250
Alistipes	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Anaerobacillus	0.000	0.000	0.000	0.000	0.002	0.006	0.002	0.005
Anaerococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Anaerofustis	0.003	0.006	0.000	0.000	0.000	0.000	0.004	0.007
Anaerostipes	0.142	0.121	0.033	0.034	0.014	0.023	0.057	0.060
Anaerotruncus	0.005	0.010	0.006	0.011	0.000	0.000	0.024	0.031
Atopobium	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.005
Bacteroides	25.528	15.032	37.395	10.619	31.893	20.803	27.708	6.211
Bifidobacterium	5.559	4.887	5.656	4.178	9.193	14.627	18.073	19.059
Bilophila	0.121	0.098	1.046	1.035	1.498	1.164	2.438	1.493
Blautia	4.769	2.950	3.912	2.688	1.982	1.377	5.943	4.212
Bulleidia	0.009	0.026	0.000	0.000	0.000	0.000	0.000	0.000
Butyricimonas	0.087	0.128	0.078	0.125	0.044	0.060	0.063	0.068
Campylobacter	0.005	0.009	0.002	0.005	0.000	0.000	0.013	0.036
Cardiobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Catenibacterium	0.101	0.285	0.002	0.005	0.000	0.000	0.000	0.000
cc_115	0.005	0.007	0.014	0.016	0.008	0.018	0.005	0.010
Christensenella	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.007
Chthoniobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Citrobacter	0.017	0.031	0.003	0.006	6.228	17.412	0.003	0.006
Clostridium	0.072	0.076	0.759	1.854	0.704	1.635	0.425	1.117
Clostridium	0.000	0.000	0.000	0.000	0.006	0.017	0.000	0.000
Clostridium	0.000	0.000	0.000	0.000	0.002	0.005	0.000	0.000
Collinsella	0.350	0.347	0.638	0.579	0.600	0.663	2.900	4.660
Coprobacillus	0.027	0.038	0.017	0.031	0.006	0.012	0.004	0.012
Coprococcus	3.192	2.537	3.017	2.610	3.873	3.829	4.553	2.948

Corynebacterium	0.000	0.000	0.002	0.006	0.004	0.007	0.004	0.011
Curvibacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dehalobacterium	0.002	0.004	0.000	0.000	0.000	0.000	0.004	0.011
Desulfovibrio	0.076	0.215	0.121	0.186	0.036	0.085	0.082	0.119
Dialister	0.298	0.307	0.588	0.934	0.402	0.511	0.365	0.562
Dorea	0.581	0.199	1.380	1.183	1.483	1.396	1.965	1.438
Eggerthella	0.038	0.058	0.081	0.140	0.060	0.123	0.097	0.130
Eikenella	0.000	0.000	0.002	0.005	0.000	0.000	0.002	0.005
Enterobacter	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Enterococcus	0.435	0.944	0.614	1.064	0.592	1.173	1.237	1.865
Epulopiscium	0.000	0.000	0.002	0.005	0.000	0.000	0.007	0.008
Erwinia	0.002	0.004	0.000	0.000	0.007	0.021	0.002	0.005
Escherichia	0.027	0.056	0.366	0.615	0.285	0.621	0.365	0.567
Faecalibacterium	3.386	1.872	4.864	3.504	2.251	1.559	0.926	1.055
Finegoldia	0.000	0.000	0.003	0.010	0.000	0.000	0.005	0.009
Flavobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacterium	0.012	0.022	0.011	0.017	7.646	21.595	1.796	5.068
Gemella	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Granulicatella	0.008	0.013	0.005	0.010	0.000	0.000	0.003	0.006
Haemophilus	0.054	0.095	0.026	0.062	0.014	0.039	0.005	0.015
Helicobacter	0.006	0.016	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.011	0.024	0.017	0.016	0.019	0.027	0.015	0.022
Klebsiella	0.057	0.162	0.011	0.032	0.009	0.020	0.000	0.000
Lachnobacterium	0.143	0.250	0.061	0.138	0.070	0.146	0.017	0.029
Lachnospira	0.988	0.660	0.500	0.547	0.240	0.277	0.416	0.335
Lactobacillus	6.196	3.676	2.417	2.031	2.337	1.861	1.375	1.182
Lactococcus	0.005	0.006	0.002	0.006	0.105	0.243	0.011	0.032
Megamonas	0.000	0.000	0.002	0.004	0.008	0.023	0.016	0.044
Megasphaera	0.011	0.017	0.010	0.024	0.014	0.041	0.282	0.741
Mesorhizobium	0.002	0.005	0.002	0.006	0.000	0.000	0.002	0.005
Methanobrevibacter	0.011	0.030	0.006	0.018	0.014	0.025	0.008	0.023
Morganella	0.000	0.000	0.012	0.033	0.096	0.154	0.028	0.063
Odoribacter	0.289	0.256	0.271	0.152	0.142	0.164	0.187	0.177
Oribacterium	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Oscillospira	0.545	0.449	1.455	0.978	1.402	1.287	2.554	2.487
Other	0.003	0.006	0.002	0.004	0.012	0.035	0.000	0.000
Parabacteroides	0.995	0.845	2.315	1.695	2.166	2.034	2.835	2.781
Paraprevotella	0.219	0.429	0.147	0.299	0.203	0.259	0.119	0.220

Pediococcus	0.009	0.017	0.002	0.005	0.007	0.010	0.000	0.000
Peptococcus	0.000	0.000	0.000	0.000	0.022	0.062	0.000	0.000
Peptostreptococcus	0.009	0.026	0.000	0.000	0.003	0.009	0.013	0.032
Phascolarctobacterium	1.055	1.431	1.918	2.147	1.441	2.102	0.918	1.048
Porphyromonas	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Prevotella	7.271	14.491	2.492	5.653	3.281	8.459	0.012	0.018
Proteus	0.005	0.009	0.004	0.007	0.002	0.006	0.005	0.007
PSB-M-3	0.000	0.000	0.000	0.000	0.002	0.005	0.001	0.004
Pseudomonas	0.000	0.000	0.000	0.000	0.004	0.010	0.000	0.000
Pyramidobacter	0.003	0.009	0.005	0.013	0.014	0.040	0.038	0.108
Ralstonia	0.002	0.004	0.006	0.012	0.000	0.000	0.000	0.000
Rikenella	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.005
Roseburia	1.358	1.944	0.432	0.530	0.149	0.244	0.110	0.100
Rothia	0.008	0.013	0.000	0.000	0.000	0.000	0.002	0.006
Ruminococcus	3.715	3.478	1.865	1.378	2.050	1.942	2.285	2.575
Scardovia	0.002	0.004	0.000	0.000	0.002	0.006	0.000	0.000
Selenomonas	0.005	0.009	0.000	0.000	0.000	0.000	0.007	0.021
Serratia	0.005	0.013	0.014	0.022	0.256	0.529	0.003	0.006
SHD-231	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Slackia	0.040	0.048	0.065	0.149	0.035	0.053	0.081	0.154
SMB53	0.025	0.046	0.022	0.046	0.015	0.018	0.015	0.021
Staphylococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Streptococcus	28.025	14.188	17.604	19.228	9.942	9.803	9.763	9.633
Sutterella	1.056	0.329	3.356	2.466	2.201	2.304	3.580	3.000
Trabulsiella	0.002	0.004	0.000	0.000	0.011	0.031	0.000	0.000
Turicibacter	0.052	0.124	0.025	0.047	0.006	0.017	0.017	0.031
Veillonella	0.322	0.469	0.675	1.347	0.385	0.846	0.884	1.393

Table A8. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon VSL#3 + lactulose treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	3.227	2.090	14.168	15.097	15.843	13.699	7.331	8.028
Bacteroidetes	37.558	7.973	25.487	13.368	26.011	11.137	27.757	11.391
Cyanobacteria	0.030	0.062	0.001	0.003	0.010	0.028	0.001	0.003
Euryarchaeota	0.005	0.016	0.007	0.019	0.013	0.028	0.013	0.041

Firmicutes	57.634	8.265	51.535	11.514	52.300	6.715	48.621	11.271
Fusobacteria	0.056	0.176	0.007	0.011	0.017	0.029	0.518	1.471
Lentisphaerae	0.006	0.011	0.000	0.000	0.000	0.000	0.012	0.035
Proteobacteria	1.392	0.677	8.760	8.002	5.770	6.154	15.614	11.100
Synergistetes	0.001	0.003	0.001	0.003	0.000	0.000	0.021	0.060
Tenericutes	0.018	0.043	0.001	0.003	0.000	0.000	0.001	0.003
TM7	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Verrucomicrobia	0.070	0.129	0.033	0.082	0.036	0.091	0.110	0.245

	Time point							
	T0		**T5**		**T10**		**T24**	
Order	**mean ± sd**		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinomycetales	0.022	0.021	0.007	0.008	0.004	0.007	0.005	0.005
Aeromonadales	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Bacillales	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Bacteroidales	37.559	7.972	25.488	13.368	26.012	11.138	27.757	11.391
Bifidobacteriales	2.420	1.771	12.474	14.682	13.507	12.484	5.935	7.363
Burkholderiales	0.835	0.357	1.401	0.964	0.724	0.459	2.248	2.258
Campylobacterales	0.007	0.015	0.001	0.003	0.001	0.003	0.002	0.004
Clostridiales	41.548	11.636	33.529	13.341	34.268	7.967	29.992	11.148
Coriobacteriales	0.785	0.408	1.687	1.891	2.332	2.463	1.391	1.447
Desulfovibrionales	0.222	0.172	0.179	0.173	0.514	0.559	1.668	0.758
Enterobacteriales	0.294	0.574	7.154	7.532	4.501	5.875	11.667	10.829
Erysipelotrichales	1.504	1.612	3.824	4.945	8.476	9.132	4.177	6.150
Fusobacteriales	0.056	0.176	0.007	0.011	0.017	0.029	0.518	1.471
Gemellales	0.004	0.009	0.000	0.000	0.002	0.004	0.002	0.004
Lactobacillales	14.529	9.576	14.174	10.400	9.543	9.770	14.434	17.147
Methanobacteriales	0.005	0.016	0.007	0.019	0.013	0.028	0.013	0.041
ML615J-28	0.010	0.032	0.000	0.000	0.000	0.000	0.000	0.000
Neisseriales	0.000	0.000	0.000	0.000	0.002	0.006	0.000	0.000
Pasteurellales	0.020	0.034	0.018	0.032	0.021	0.030	0.023	0.031
RF32	0.010	0.018	0.006	0.011	0.005	0.016	0.004	0.009
RF39	0.008	0.018	0.001	0.003	0.000	0.000	0.001	0.003
Rhizobiales	0.003	0.007	0.000	0.000	0.000	0.000	0.001	0.003
SHA-98	0.003	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Synergistales	0.001	0.003	0.001	0.003	0.000	0.000	0.021	0.060
Turicibacterales	0.045	0.095	0.007	0.011	0.011	0.023	0.017	0.028
Verrucomicrobiales	0.070	0.129	0.033	0.082	0.035	0.091	0.110	0.245

Victivallales	0.006	0.011	0.000	0.000	0.000	0.000	0.012	0.035
YS2	0.030	0.062	0.001	0.003	0.010	0.028	0.001	0.003

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.432	0.363	0.187	0.192	0.220	0.194	0.399	0.452
[Cerasicoccaceae]	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
[Mogibacteriaceae]	0.116	0.112	0.150	0.216	0.102	0.178	0.116	0.201
[Odoribacteraceae]	0.387	0.180	0.197	0.213	0.159	0.168	0.252	0.192
[Paraprevotellaceae]	0.809	1.464	0.168	0.306	0.237	0.500	0.170	0.343
[Tissierellaceae]	0.000	0.000	0.003	0.005	0.013	0.028	0.034	0.069
Actinomycetaceae	0.011	0.019	0.006	0.009	0.003	0.007	0.001	0.003
Alcaligenaceae	0.866	0.374	1.436	0.977	0.753	0.484	2.291	2.294
Bacteroidaceae	28.237	12.917	19.671	11.847	21.125	7.818	23.057	10.830
Bifidobacteriaceae	2.539	1.861	12.832	14.859	14.070	13.052	6.090	7.689
Campylobacteraceae	0.000	0.000	0.001	0.003	0.001	0.003	0.002	0.004
Carnobacteriaceae	0.013	0.025	0.001	0.003	0.002	0.004	0.002	0.004
Christensenellaceae	0.085	0.196	0.054	0.091	0.022	0.025	0.006	0.009
Clostridiaceae	0.420	0.453	0.667	0.708	0.320	0.292	0.633	0.772
Comamonadaceae	0.000	0.000	0.002	0.004	0.000	0.000	0.000	0.000
Coriobacteriaceae	0.825	0.434	1.773	2.017	2.456	2.621	1.452	1.537
Corynebacteriaceae	0.003	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Dehalobacteriaceae	0.002	0.007	0.001	0.003	0.001	0.003	0.000	0.000
Desulfovibrionaceae	0.235	0.186	0.186	0.180	0.538	0.598	1.712	0.781
Dethiosulfovibrionaceae	0.001	0.003	0.001	0.003	0.000	0.000	0.020	0.063
Enterobacteriaceae	0.305	0.593	7.314	7.646	4.712	6.310	11.946	11.066
Enterococcaceae	0.513	1.030	2.300	3.067	1.478	2.485	0.878	1.643
Erysipelotrichaceae	1.600	1.772	3.893	5.005	8.672	9.362	4.237	6.171
Eubacteriaceae	0.000	0.000	0.002	0.004	0.001	0.003	0.017	0.045
Fusobacteriaceae	0.056	0.178	0.007	0.011	0.017	0.029	0.523	1.483
Gemellaceae	0.004	0.009	0.000	0.000	0.002	0.004	0.002	0.004
Helicobacteraceae	0.007	0.016	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	18.923	4.676	14.724	7.082	17.146	4.648	12.119	6.542
Lactobacillaceae	3.517	4.357	3.124	2.946	1.187	1.581	3.573	4.860
Leuconostocaceae	0.024	0.047	0.014	0.026	0.004	0.010	0.024	0.058
Methanobacteriaceae	0.005	0.017	0.007	0.020	0.014	0.029	0.014	0.043
Microbacteriaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.002	0.004

Micrococcaceae	0.007	0.010	0.000	0.000	0.001	0.003	0.002	0.004
Oxalobacteraceae	0.002	0.005	0.000	0.000	0.000	0.000	0.002	0.007
Pasteurellaceae	0.021	0.035	0.018	0.032	0.022	0.030	0.024	0.032
Peptococcaceae	0.000	0.000	0.148	0.467	0.195	0.612	0.000	0.000
Peptostreptococcaceae	0.020	0.032	0.013	0.015	0.003	0.010	0.023	0.035
Phyllobacteriaceae	0.003	0.007	0.000	0.000	0.000	0.000	0.001	0.003
Porphyromonadaceae	1.352	0.946	1.475	2.219	1.513	1.776	2.210	1.715
Prevotellaceae	5.852	12.023	3.488	9.434	2.389	3.963	1.104	2.444
Propionibacteriaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Rikenellaceae	1.784	1.641	1.107	1.309	1.059	0.616	0.951	0.972
Ruminococcaceae	17.688	8.001	14.094	7.321	11.955	5.859	10.616	6.088
S24-7	0.263	0.578	0.002	0.007	0.188	0.337	0.260	0.431
Staphylococcaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Streptococcaceae	10.925	7.321	9.121	9.384	7.164	8.795	10.139	16.234
Succinivibrionaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Synergistaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004
Turicibacteraceae	0.050	0.106	0.007	0.011	0.012	0.025	0.018	0.030
Veillonellaceae	2.011	1.360	1.769	1.060	2.205	0.634	4.949	5.803
Verrucomicrobiaceae	0.074	0.136	0.035	0.087	0.037	0.096	0.114	0.257
Victivallaceae	0.006	0.011	0.000	0.000	0.000	0.000	0.013	0.036

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.945	1.929	1.536	3.407	1.355	1.766	1.792	2.597
[Prevotella]	0.642	1.628	0.123	0.262	0.254	0.662	0.183	0.470
[Ruminococcus]	1.126	0.680	1.297	0.853	0.894	0.695	0.675	0.429
Acidaminococcus	0.285	0.498	0.116	0.209	0.162	0.256	1.824	4.521
Acinetobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Actinobacillus	0.000	0.000	0.001	0.004	0.007	0.022	0.001	0.004
Actinomyces	0.014	0.022	0.009	0.013	0.003	0.008	0.001	0.004
Adlercreutzia	0.068	0.107	0.073	0.120	0.008	0.014	0.003	0.006
Akkermansia	0.097	0.178	0.041	0.102	0.045	0.111	0.156	0.348
Anaerococcus	0.000	0.000	0.001	0.005	0.008	0.018	0.006	0.014
Anaerofustis	0.000	0.000	0.003	0.006	0.001	0.004	0.003	0.011
Anaerostipes	0.131	0.123	0.090	0.080	0.045	0.058	0.062	0.122
Anaerotruncus	0.006	0.014	0.009	0.016	0.004	0.007	0.005	0.009
Atopobium	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000

Bacteroides	36.49	15.73	26.62	16.12	31.58	14.23	32.00	14.20
Bifidobacterium	3.359	2.522	17.22	20.83	18.419	16.675	8.194	9.620
Bilophila	0.230	0.124	0.215	0.216	0.819	1.083	2.300	1.273
Blautia	6.059	2.833	5.651	3.182	5.038	3.083	2.202	1.716
Bulleidia	0.014	0.045	0.035	0.110	0.000	0.000	0.026	0.083
Butyricimonas	0.130	0.136	0.090	0.179	0.100	0.162	0.120	0.150
Campylobacter	0.000	0.000	0.001	0.004	0.002	0.005	0.003	0.006
Catenibacterium	0.099	0.314	0.776	2.455	0.002	0.005	0.156	0.482
cc_115	0.096	0.218	0.005	0.013	0.003	0.005	0.009	0.023
Christensenella	0.006	0.010	0.003	0.006	0.001	0.004	0.001	0.004
Citrobacter	0.016	0.036	0.427	1.314	0.245	0.774	1.769	4.713
Clostridium	0.047	0.044	0.345	0.809	0.132	0.216	0.429	1.084
Collinsella	0.571	0.573	1.806	2.491	2.856	3.157	1.259	1.517
Coprobacillus	0.015	0.022	0.004	0.007	0.001	0.004	0.005	0.008
Coprococcus	5.147	2.746	4.599	3.354	5.701	3.654	4.336	4.030
Corynebacterium	0.004	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Cryocola	0.000	0.000	0.001	0.005	0.000	0.000	0.001	0.004
Dehalobacterium	0.003	0.009	0.002	0.006	0.001	0.004	0.000	0.000
Delftia	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Desulfovibrio	0.067	0.212	0.033	0.076	0.015	0.036	0.133	0.257
Dialister	0.578	0.819	0.570	0.752	0.838	1.133	0.335	0.735
Dorea	0.854	0.400	0.897	0.539	1.388	1.377	1.885	2.210
Eggerthella	0.011	0.016	0.043	0.070	0.051	0.066	0.109	0.145
Eikenella	0.000	0.000	0.000	0.000	0.003	0.010	0.000	0.000
Enterobacter	0.001	0.004	0.000	0.000	0.001	0.004	0.005	0.010
Enterococcus	0.716	1.465	2.388	3.733	1.894	3.303	0.889	2.034
Epulopiscium	0.000	0.000	0.004	0.011	0.004	0.012	0.008	0.020
Erwinia	0.000	0.000	0.000	0.000	0.003	0.006	0.003	0.008
Escherichia	0.008	0.011	0.322	0.476	0.225	0.394	0.389	0.503
Faecalibacterium	3.665	1.651	2.716	2.012	2.108	1.881	0.976	1.265
Finegoldia	0.000	0.000	0.001	0.004	0.006	0.009	0.023	0.046
Fusobacterium	0.065	0.204	0.009	0.014	0.024	0.040	0.600	1.641
Granulicatella	0.015	0.031	0.001	0.004	0.002	0.005	0.001	0.004
Haemophilus	0.025	0.040	0.023	0.041	0.025	0.023	0.034	0.047
Helicobacter	0.006	0.015	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.023	0.042	0.018	0.028	0.015	0.017	0.053	0.096
Klebsiella	0.059	0.185	0.075	0.219	0.011	0.035	0.172	0.513
Lachnobacterium	0.228	0.298	0.057	0.106	0.040	0.084	0.030	0.090

Lachnospira	1.186	0.926	0.341	0.412	0.154	0.157	0.344	0.391
Lactobacillus	4.145	4.957	3.629	3.467	1.321	1.779	4.555	6.545
Lactococcus	0.010	0.023	0.003	0.008	0.001	0.004	0.040	0.119
Leuconostoc	0.015	0.037	0.014	0.034	0.002	0.005	0.001	0.004
Luteolibacter	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Megamonas	0.070	0.223	0.000	0.000	0.000	0.000	0.227	0.718
Megasphaera	0.093	0.289	0.004	0.013	0.006	0.012	1.389	4.241
Mesorhizobium	0.005	0.010	0.000	0.000	0.000	0.000	0.002	0.007
Methanobrevibacter	0.007	0.022	0.009	0.025	0.017	0.036	0.018	0.058
Microbacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Mogibacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.007
Morganella	0.000	0.000	0.047	0.148	0.025	0.069	0.040	0.090
Moryella	0.001	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Odoribacter	0.381	0.177	0.183	0.175	0.143	0.147	0.252	0.236
Oscillospira	0.908	0.753	1.641	2.126	1.526	1.381	2.104	0.928
Other	0.001	0.004	0.003	0.009	0.000	0.000	0.000	0.000
Oxalobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.013
p-75-a5	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Parabacteroides	1.789	1.300	1.982	2.889	2.351	2.675	3.289	2.827
Paraprevotella	0.310	0.585	0.082	0.175	0.091	0.160	0.077	0.138
Pediococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Peptococcus	0.000	0.000	0.188	0.596	0.256	0.803	0.000	0.000
Peptoniphilus	0.000	0.000	0.001	0.004	0.006	0.019	0.018	0.034
Peptostreptococcus	0.009	0.030	0.000	0.000	0.000	0.000	0.011	0.036
Phascolarctobacterium	1.157	1.378	1.092	1.229	1.270	1.107	1.944	2.090
Prevotella	6.956	13.907	4.153	11.110	4.120	6.870	1.714	3.882
Proteus	0.000	0.000	0.004	0.007	0.000	0.000	0.004	0.009
Pseudoramibacter_Eubacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.057
Pyramidobacter	0.001	0.004	0.001	0.004	0.000	0.000	0.027	0.085
Ralstonia	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Rikenella	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.005
Roseburia	1.168	2.025	0.206	0.196	0.107	0.115	0.086	0.139
Rothia	0.009	0.012	0.000	0.000	0.001	0.004	0.003	0.006
Ruminococcus	4.580	4.710	3.744	3.388	3.047	2.057	2.811	2.730
Scardovia	0.001	0.004	0.000	0.000	0.000	0.000	0.001	0.005
Selenomonas	0.000	0.000	0.001	0.004	0.006	0.015	0.001	0.004
Serratia	0.006	0.016	0.017	0.055	0.005	0.013	0.802	2.507
Slackia	0.074	0.101	0.063	0.132	0.015	0.024	0.019	0.053

SMB53	0.048	0.094	0.008	0.015	0.023	0.034	0.041	0.074
Sphingobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Staphylococcus	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Streptococcus	13.69	8.903	11.819	12.074	9.194	11.084	12.917	20.793
Sutterella	1.063	0.477	1.949	1.384	1.023	0.550	3.124	2.983
Tetragenococcus	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Trabulsiella	0.000	0.000	0.001	0.004	0.000	0.000	0.008	0.025
Turicibacter	0.068	0.148	0.010	0.015	0.018	0.040	0.029	0.051
Veillonella	0.307	0.486	0.529	1.112	0.931	1.635	0.901	1.372
WAL_1855D	0.000	0.000	0.000	0.000	0.002	0.006	0.000	0.000

Table A9. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon VSL#3 + lactulose + rifaximin treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	4.249	3.335	3.324	3.473	5.362	5.697	4.838	8.990
Bacteroidetes	34.848	13.123	31.679	11.952	20.894	14.725	14.264	16.806
Chloroflexi	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Cyanobacteria	0.021	0.042	0.015	0.034	0.001	0.003	0.000	0.000
Euryarchaeota	0.003	0.010	0.004	0.013	0.006	0.019	0.049	0.153
Firmicutes	54.038	10.280	50.331	18.508	64.218	16.461	67.986	12.988
Fusobacteria	0.010	0.013	1.363	4.263	1.186	3.227	1.465	3.197
Lentisphaerae	0.001	0.003	0.002	0.004	0.000	0.000	0.003	0.010
Proteobacteria	6.779	9.846	13.228	17.303	8.269	6.737	11.347	11.967
Synergistetes	0.001	0.003	0.002	0.006	0.003	0.010	0.010	0.032
Tenericutes	0.000	0.000	0.005	0.016	0.000	0.000	0.001	0.003
TM7	0.002	0.004	0.002	0.004	0.000	0.000	0.000	0.000
Verrucomicrobia	0.047	0.063	0.045	0.071	0.060	0.130	0.036	0.104

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Cerasicoccales]	0,0010	0,0032	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
[Chthoniobacterales]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0010	0,0032
Actinomycetales	0,0242	0,0203	0,0067	0,0116	0,0051	0,0072	0,0080	0,0148

Aeromonadales	0,0000	0,0000	0,0000	0,0000	0,0010	0,0032	0,0000	0,0000
Anaerolineales	0,0000	0,0000	0,0000	0,0000	0,0010	0,0032	0,0000	0,0000
Bacillales	0,0040	0,0070	0,0000	0,0000	0,0010	0,0032	0,0000	0,0000
Bacteroidales	34,8493	13,1219	23,6641	4,9120	20,8944	14,7253	14,2635	16,8067
Bifidobacteriales	3,6759	3,3896	2,7386	1,6089	4,7268	5,8875	4,6418	8,9814
Burkholderiales	0,8106	0,4240	0,5110	0,6463	1,9035	1,4214	2,3558	1,5511
Campylobacterales	0,0161	0,0273	0,0067	0,0116	0,0041	0,0098	0,0101	0,0178
Clostridiales	33,4203	12,8815	27,8670	15,1197	27,7613	13,0436	17,8255	13,9702
Coriobacteriales	0,5490	0,3713	0,5993	0,3059	0,6304	0,5194	0,1882	0,1928
CW040	0,0010	0,0032	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
Desulfovibrionales	0,1571	0,1221	1,6965	1,8621	1,1162	0,6943	1,3845	0,9219
Enterobacteriales	5,6162	9,6830	16,9283	19,2348	5,2172	5,9691	7,5920	11,8735
Erysipelotrichales	1,0828	0,6822	21,7571	18,4035	21,2751	23,5212	34,8289	19,1989
Flavobacteriales	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0010	0,0032
Fusobacteriales	0,0101	0,0126	0,0000	0,0000	1,1856	3,2272	1,4649	3,1974
Gemellales	0,0151	0,0219	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
Lactobacillales	19,5100	19,0727	4,0766	1,5651	15,1668	12,7784	15,3209	11,3722
Methanobacteriales	0,0030	0,0096	0,0439	0,0760	0,0061	0,0192	0,0494	0,1527
Neisseriales	0,0020	0,0043	0,0000	0,0000	0,0010	0,0032	0,0000	0,0000
Pasteurellales	0,1614	0,2697	0,0000	0,0000	0,0232	0,0735	0,0020	0,0043
Pseudomonadales	0,0010	0,0032	0,0000	0,0000	0,0010	0,0032	0,0000	0,0000
RF32	0,0091	0,0220	0,0101	0,0175	0,0020	0,0042	0,0000	0,0000
RF39	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0010	0,0032
Rhizobiales	0,0030	0,0049	0,0067	0,0058	0,0000	0,0000	0,0000	0,0000
SHA-98	0,0000	0,0000	0,0000	0,0000	0,0010	0,0032	0,0000	0,0000
Synergistales	0,0010	0,0032	0,0000	0,0000	0,0030	0,0096	0,0101	0,0319
Turicibacterales	0,0081	0,0149	0,0638	0,1106	0,0126	0,0332	0,0131	0,0345
Verrucomicrobiales	0,0464	0,0605	0,0202	0,0202	0,0596	0,1299	0,0353	0,1047
Victivallales	0,0010	0,0032	0,0000	0,0000	0,0000	0,0000	0,0030	0,0095
YS2	0,0212	0,0415	0,0034	0,0058	0,0010	0,0032	0,0000	0,0000

	Time point							
Family	**T0 mean ± sd**		**T5 mean ± sd**		**T10 mean ± sd**		**T24 mean ± sd**	
[Barnesiellaceae]	0.356	0.390	0.255	0.299	0.085	0.076	0.134	0.330
[Cerasicoccaceae]	0.001	0.003	0.001	0.003	0.000	0.000	0.000	0.000
[Chthoniobacteraceae]	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
[Mogibacteriaceae]	0.039	0.038	0.034	0.054	0.111	0.281	0.020	0.039
[Odoribacteraceae]	0.287	0.242	0.264	0.201	0.110	0.122	0.038	0.059
[Paraprevotellaceae]	0.963	1.880	0.689	1.099	0.116	0.180	0.037	0.101

[Tissierellaceae]	0.002	0.004	0.004	0.009	0.005	0.016	0.054	0.160
Actinomycetaceae	0.013	0.017	0.013	0.020	0.002	0.004	0.005	0.011
Aerococcaceae	0.003	0.005	0.001	0.003	0.001	0.003	0.000	0.000
Alcaligenaceae	0.833	0.446	1.602	1.073	1.931	1.429	2.377	1.576
Anaerolinaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Bacillaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Bacteroidaceae	27.366	11.476	23.173	11.289	15.179	10.934	11.504	14.893
Bifidobacteriaceae	3.752	3.427	2.864	3.391	4.783	5.921	4.663	9.018
Campylobacteraceae	0.003	0.005	0.005	0.009	0.004	0.010	0.010	0.018
Carnobacteriaceae	0.020	0.034	0.009	0.009	0.003	0.005	0.004	0.007
Christensenellaceae	0.009	0.012	0.010	0.024	0.035	0.099	0.025	0.060
Clostridiaceae	0.363	0.347	0.413	0.355	0.817	1.278	0.252	0.398
Comamonadaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Coriobacteriaceae	0.566	0.385	0.518	0.358	0.648	0.539	0.191	0.199
Corynebacteriaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.001	0.003
Dehalobacteriaceae	0.001	0.003	0.000	0.000	0.002	0.007	0.000	0.000
Desulfovibrionaceae	0.162	0.127	1.038	0.738	1.137	0.712	1.403	0.963
Dethiosulfovibrionaceae	0.001	0.003	0.002	0.006	0.003	0.010	0.010	0.032
Enterobacteriaceae	5.713	9.869	10.724	17.151	5.328	6.054	7.634	11.906
Enterococcaceae	0.581	0.790	0.299	0.538	0.250	0.335	1.830	4.770
Erysipelotrichaceae	1.106	0.690	9.547	12.370	21.534	23.783	34.955	19.269
Eubacteriaceae	0.001	0.003	0.000	0.000	0.003	0.010	0.000	0.000
Flavobacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Fusobacteriaceae	0.009	0.011	1.378	4.312	1.194	3.252	1.469	3.207
Gemellaceae	0.015	0.022	0.006	0.014	0.000	0.000	0.000	0.000
Helicobacteraceae	0.014	0.030	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	15.309	5.505	9.656	3.292	10.028	6.995	6.023	3.494
Lactobacillaceae	4.047	4.629	2.659	3.131	3.375	3.486	6.097	8.248
Leptotrichiaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Leuconostocaceae	0.017	0.050	0.024	0.067	0.006	0.017	0.011	0.029
Methanobacteriaceae	0.003	0.010	0.004	0.013	0.006	0.019	0.050	0.153
Micrococcaceae	0.011	0.019	0.006	0.011	0.002	0.004	0.002	0.004
Neisseriaceae	0.002	0.004	0.002	0.004	0.001	0.003	0.000	0.000
Nocardioidaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Oxalobacteraceae	0.001	0.003	0.001	0.003	0.000	0.000	0.000	0.000
Pasteurellaceae	0.164	0.275	0.038	0.106	0.024	0.074	0.002	0.004
Peptococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003
Peptostreptococcaceae	0.003	0.007	0.007	0.016	0.008	0.016	0.025	0.073

Phyllobacteriaceae	0.003	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Porphyromonadaceae	0.933	0.759	1.258	1.204	0.713	0.820	1.164	3.322
Prevotellaceae	3.587	7.500	4.599	6.918	2.447	5.250	1.224	3.853
Propionibacteriaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Pseudomonadaceae	0.001	0.003	0.001	0.003	0.001	0.003	0.000	0.000
Rikenellaceae	1.074	0.823	0.813	0.869	0.874	1.280	0.256	0.502
Ruminococcaceae	14.359	7.972	16.459	8.970	13.035	12.024	10.589	12.558
S24-7	1.228	3.402	1.344	3.351	1.808	5.422	0.004	0.009
Streptococcaceae	15.153	17.023	7.972	8.276	11.722	11.465	7.509	9.304
Succinivibrionaceae	0.000	0.000	0.001	0.003	0.001	0.003	0.000	0.000
Turicibacteraceae	0.008	0.015	0.027	0.083	0.013	0.033	0.013	0.036
Veillonellaceae	1.859	1.123	2.232	1.370	2.591	4.262	0.372	0.359
Verrucomicrobiaceae	0.048	0.062	0.045	0.073	0.061	0.132	0.035	0.105
Victivallaceae	0.001	0.003	0.002	0.004	0.000	0.000	0.003	0.010

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.146	0.207	2.578	3.993	2.795	4.454	5.730	6.089
[Prevotella]	0.976	2.100	0.823	1.626	0.086	0.247	0.002	0.007
[Ruminococcus]	1.019	0.895	0.857	0.903	0.644	0.519	1.881	4.159
Acidaminococcus	0.178	0.282	0.105	0.179	0.067	0.139	0.023	0.045
Actinobacillus	0.005	0.008	0.005	0.017	0.000	0.000	0.000	0.000
Actinomyces	0.022	0.031	0.026	0.048	0.003	0.006	0.007	0.015
Adlercreutzia	0.042	0.073	0.038	0.068	0.062	0.096	0.032	0.072
Aggregatibacter	0.004	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Akkermansia	0.062	0.080	0.067	0.101	0.106	0.205	0.052	0.148
Anaerococcus	0.000	0.000	0.004	0.008	0.000	0.000	0.003	0.009
Anaerostipes	0.076	0.073	0.054	0.052	0.046	0.057	0.018	0.031
Anaerotruncus	0.006	0.008	0.003	0.006	0.011	0.029	0.000	0.000
Arthrobacter	0.000	0.000	0.002	0.005	0.000	0.000	0.000	0.000
Atopobium	0.003	0.007	0.001	0.004	0.000	0.000	0.000	0.000
Bacteroides	38.93	17.231	35.150	15.486	24.601	15.297	18.871	22.734
Bifidobacterium	5.043	3.999	4.586	5.013	8.678	9.764	7.968	12.824
Bilophila	0.193	0.169	1.454	1.066	2.210	2.373	2.432	1.511
Blautia	4.402	2.351	3.642	1.061	3.152	2.402	1.269	0.995
Bulleidia	0.002	0.006	0.052	0.158	0.117	0.371	0.134	0.404
Butyricimonas	0.070	0.099	0.130	0.166	0.040	0.057	0.010	0.017

Campylobacter	0.005	0.008	0.010	0.016	0.006	0.013	0.018	0.033
Catenibacterium	0.000	0.000	2.744	8.659	5.675	17.94	5.979	18.88
cc_115	0.015	0.035	0.024	0.062	0.034	0.072	0.024	0.052
Christensenella	0.000	0.000	0.002	0.005	0.000	0.000	0.001	0.005
Chthoniobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Citrobacter	0.014	0.045	3.116	9.819	0.128	0.364	5.643	14.12
Clostridium	0.069	0.060	0.165	0.216	0.744	1.762	0.328	0.782
Clostridium	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Clostridium	0.000	0.000	0.003	0.006	0.006	0.015	0.005	0.009
Collinsella	0.529	0.416	0.426	0.312	0.690	0.904	0.181	0.256
Coprobacillus	0.007	0.012	0.019	0.024	0.033	0.045	0.057	0.106
Coprococcus	4.035	2.927	1.932	1.310	2.578	2.296	0.647	0.578
Corynebacterium	0.000	0.000	0.001	0.004	0.000	0.000	0.001	0.004
Dehalobacterium	0.001	0.004	0.000	0.000	0.003	0.010	0.000	0.000
Desulfovibrio	0.024	0.063	0.056	0.140	0.137	0.281	0.132	0.275
Dialister	0.108	0.184	0.607	1.098	0.627	0.800	0.290	0.571
Dorea	0.773	0.440	0.669	0.389	1.536	1.983	0.801	1.359
Eggerthella	0.045	0.068	0.028	0.052	0.069	0.110	0.009	0.012
Eikenella	0.001	0.004	0.004	0.009	0.001	0.004	0.000	0.000
Enterobacter	0.000	0.000	0.005	0.011	0.001	0.004	0.010	0.027
Enterococcus	0.895	1.212	0.648	1.254	0.636	1.257	4.146	10.56
Epulopiscium	0.002	0.006	0.002	0.005	0.006	0.010	0.000	0.000
Erwinia	0.002	0.006	0.004	0.009	0.000	0.000	0.002	0.008
Escherichia	0.190	0.369	0.283	0.589	0.254	0.310	0.172	0.363
Facklamia	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Faecalibacterium	3.522	2.394	4.501	2.691	2.702	2.604	0.756	1.101
Finegoldia	0.004	0.008	0.000	0.000	0.002	0.007	0.000	0.000
Flavobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.010
Fusobacterium	0.014	0.018	1.554	4.834	1.456	3.651	2.439	5.145
Gemella	0.006	0.008	0.004	0.009	0.000	0.000	0.000	0.000
Granulicatella	0.025	0.043	0.014	0.013	0.004	0.006	0.006	0.010
Haemophilus	0.277	0.513	0.086	0.256	0.031	0.098	0.004	0.009
Helicobacter	0.019	0.040	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.004	0.009	0.035	0.029	0.027	0.036	0.046	0.046
Janthinobacterium	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Kingella	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Klebsiella	0.000	0.000	0.131	0.340	0.069	0.212	0.021	0.066
Lachnobacterium	0.186	0.370	0.069	0.186	0.053	0.146	0.044	0.112

Lachnospira	0.721	0.561	0.549	0.417	0.347	0.280	0.413	0.731
Lactobacillus	5.456	7.220	3.326	3.580	4.805	4.758	8.928	11.10
Lactococcus	0.008	0.018	0.021	0.050	0.010	0.025	0.124	0.304
Leptotrichia	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Leuconostoc	0.001	0.004	0.003	0.006	0.009	0.025	0.003	0.010
Megamonas	0.000	0.000	0.048	0.153	0.182	0.576	0.004	0.013
Megasphaera	0.013	0.023	0.319	0.971	1.072	3.346	0.006	0.011
Melissococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.015
Mesorhizobium	0.005	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Methanobrevibacter	0.003	0.011	0.005	0.017	0.009	0.030	0.070	0.216
Mogibacterium	0.000	0.000	0.000	0.000	0.001	0.004	0.000	0.000
Morganella	0.002	0.005	0.034	0.094	0.000	0.000	0.413	1.144
Odoribacter	0.312	0.285	0.272	0.188	0.130	0.160	0.050	0.074
Oscillospira	0.509	0.264	2.157	1.303	2.515	2.483	4.656	4.496
Other	0.001	0.004	0.000	0.000	0.002	0.005	0.000	0.000
p-75-a5	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Parabacteroides	1.212	0.985	1.783	1.727	1.193	1.343	1.651	4.646
Paraprevotella	0.208	0.339	0.172	0.262	0.090	0.177	0.051	0.142
Pediococcus	0.001	0.004	0.001	0.004	0.002	0.005	0.014	0.032
Peptoniphilus	0.000	0.000	0.002	0.005	0.003	0.011	0.164	0.514
Peptostreptococcus	0.000	0.000	0.005	0.015	0.006	0.018	0.033	0.103
Phascolarctobacterium	1.190	1.683	1.553	1.759	1.192	1.312	0.285	0.433
Porphyromonas	0.000	0.000	0.003	0.010	0.000	0.000	0.000	0.000
Prevotella	4.432	9.245	6.379	9.560	4.840	10.46	2.554	8.053
Proteus	0.006	0.013	0.000	0.000	0.007	0.022	0.005	0.011
PSB-M-3	0.000	0.000	0.001	0.004	0.003	0.011	0.001	0.004
Pseudomonas	0.001	0.005	0.001	0.005	0.002	0.006	0.000	0.000
Pseudoramibacter_Eubacterium	0.002	0.006	0.000	0.000	0.003	0.011	0.000	0.000
Pyramidobacter	0.001	0.004	0.003	0.009	0.005	0.015	0.014	0.045
Ralstonia	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Roseburia	0.452	0.450	0.155	0.248	0.234	0.349	0.024	0.040
Rothia	0.015	0.024	0.007	0.014	0.003	0.005	0.005	0.011
Ruminococcus	2.183	1.682	1.734	1.485	1.755	2.210	1.325	1.967
Scardovia	0.000	0.000	0.002	0.008	0.000	0.000	0.002	0.007
Selenomonas	0.004	0.008	0.003	0.008	0.001	0.004	0.006	0.018
Serratia	0.004	0.009	0.031	0.071	0.074	0.212	0.877	2.560
SHD-231	0.000	0.000	0.000	0.000	0.002	0.006	0.000	0.000
Slackia	0.021	0.033	0.015	0.021	0.098	0.136	0.006	0.007

SMB53	0.008	0.014	0.024	0.053	0.006	0.012	0.016	0.037
Staphylococcus	0.007	0.014	0.000	0.000	0.000	0.000	0.000	0.000
Streptococcus	18.90	19.45	11.53	10.99	17.96	14.97	13.44	16.23
Sutterella	1.153	0.600	2.497	2.445	2.835	2.001	4.595	3.971
Tetragenococcus	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Trabulsiella	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.008
Turicibacter	0.010	0.018	0.039	0.115	0.021	0.054	0.020	0.050
Veillonella	1.190	2.077	0.604	1.418	0.451	0.952	0.033	0.047

Table A10. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Cirrhotic patients (CP) upon VSL#3 + rifaximin treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	5.867	4.093	4.364	5.183	5.336	5.661	10.160	11.321
Bacteroidetes	33.537	14.661	25.386	11.472	24.852	11.057	18.090	12.357
Cyanobacteria	0.025	0.069	0.000	0.000	0.011	0.019	0.001	0.003
Euryarchaeota	0.013	0.035	0.005	0.016	0.004	0.013	0.047	0.083
Firmicutes	57.973	10.553	60.962	13.406	57.555	10.059	61.102	14.474
Fusobacteria	0.052	0.159	1.183	3.740	2.340	6.153	0.368	1.164
Lentisphaerae	0.000	0.000	0.005	0.010	0.004	0.007	0.004	0.010
Proteobacteria	2.479	3.093	8.082	9.112	9.825	6.540	10.154	15.033
Synergistetes	0.006	0.019	0.000	0.000	0.004	0.013	0.018	0.058
Tenericutes	0.007	0.022	0.002	0.006	0.005	0.010	0.005	0.011
TM7	0.000	0.000	0.000	0.000	0.002	0.004	0.002	0.006
Verrucomicrobia	0.040	0.114	0.011	0.020	0.063	0.173	0.048	0.151

	Time points							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Cerasicoccales]	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Actinomycetales	0.022	0.022	0.030	0.023	0.017	0.013	0.015	0.033
Aeromonadales	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Bacillales	0.002	0.006	0.001	0.003	0.001	0.003	0.001	0.003
Bacteroidales	33.538	14.661	25.387	11.471	24.849	11.061	18.090	12.358
Bifidobacteriales	5.012	4.279	3.796	5.316	4.909	5.779	9.634	11.444
Burkholderiales	0.904	0.413	1.816	1.693	2.390	1.650	2.000	3.036

Campylobacterales	0.002	0.004	0.008	0.010	0.009	0.022	0.004	0.007
Cardiobacteriales	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Clostridiales	36.308	12.091	34.322	13.525	27.886	12.107	34.626	17.494
Coriobacteriales	0.833	0.755	0.537	0.363	0.410	0.217	0.511	0.391
CW040	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Desulfovibrionales	0.202	0.188	0.553	0.466	2.068	1.499	2.259	2.580
Enterobacteriales	1.306	3.180	5.652	8.483	5.326	7.415	5.880	12.120
Erysipelotrichales	1.464	1.300	11.095	14.551	2.209	2.263	8.958	13.705
Flavobacteriales	0.000	0.000	0.000	0.000	0.002	0.004	0.000	0.000
Fusobacteriales	0.052	0.159	1.183	3.740	2.340	6.153	0.368	1.164
Gemellales	0.005	0.010	0.002	0.004	0.002	0.004	0.002	0.004
Lactobacillales	20.143	19.243	15.508	13.246	27.405	13.327	17.497	14.863
Methanobacteriales	0.013	0.035	0.005	0.016	0.004	0.013	0.047	0.083
ML615J-28	0.006	0.019	0.001	0.003	0.001	0.003	0.000	0.000
Neisseriales	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Pasteurellales	0.052	0.128	0.037	0.108	0.016	0.028	0.006	0.016
Pseudomonadales	0.002	0.006	0.000	0.000	0.002	0.004	0.000	0.000
RF32	0.009	0.016	0.010	0.023	0.009	0.025	0.002	0.004
RF39	0.001	0.003	0.001	0.003	0.004	0.007	0.005	0.011
Rhizobiales	0.001	0.003	0.000	0.000	0.004	0.007	0.003	0.007
SHA-98	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Sphingobacteriales	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Streptophyta	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Synergistales	0.006	0.019	0.000	0.000	0.004	0.013	0.018	0.058
Turicibacterales	0.051	0.097	0.035	0.074	0.052	0.104	0.019	0.042
Verrucomicrobiales	0.040	0.114	0.011	0.020	0.062	0.170	0.048	0.151
Victivallales	0.000	0.000	0.005	0.010	0.004	0.007	0.004	0.010
YS2	0.023	0.070	0.000	0.000	0.011	0.019	0.001	0.003

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.228	0.367	0.255	0.287	0.289	0.376	0.173	0.190
[Cerasicoccaceae]	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
[Mogibacteriaceae]	0.086	0.120	0.089	0.142	0.088	0.167	0.157	0.219
[Odoribacteraceae]	0.294	0.288	0.287	0.242	0.271	0.253	0.209	0.228
[Paraprevotellaceae]	1.015	1.709	0.232	0.446	0.579	1.043	0.099	0.149
[Tissierellaceae]	0.000	0.000	0.004	0.010	0.010	0.021	0.004	0.010

[Weeksellaceae]	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Actinomycetaceae	0.015	0.019	0.025	0.024	0.011	0.012	0.009	0.017
Aerococcaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Alcaligenaceae	0.934	0.430	1.872	1.770	2.437	1.694	2.025	3.065
Bacillaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Bacteroidaceae	23.230	12.672	19.656	11.517	18.974	10.651	14.503	10.590
Bifidobacteriaceae	5.182	4.400	3.867	5.351	4.978	5.807	9.761	11.591
Campylobacteraceae	0.002	0.004	0.005	0.007	0.006	0.013	0.004	0.007
Cardiobacteriaceae	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.000
Carnobacteriaceae	0.011	0.015	0.009	0.013	0.012	0.016	0.004	0.007
Christensenellaceae	0.050	0.132	0.046	0.103	0.040	0.103	0.126	0.256
Clostridiaceae	0.940	1.162	1.236	2.592	1.139	2.682	0.804	1.451
Coriobacteriaceae	0.869	0.796	0.553	0.374	0.420	0.224	0.524	0.406
Corynebacteriaceae	0.000	0.000	0.001	0.003	0.002	0.006	0.000	0.000
Dehalobacteriaceae	0.005	0.011	0.000	0.000	0.003	0.007	0.000	0.000
Desulfovibrionaceae	0.210	0.196	0.570	0.486	2.115	1.541	2.295	2.601
Dethiosulfovibrionaceae	0.006	0.020	0.000	0.000	0.004	0.013	0.018	0.058
Enterobacteriaceae	1.324	3.207	5.784	8.592	5.421	7.520	5.926	12.159
Enterococcaceae	0.041	0.051	0.297	0.481	0.552	0.992	0.409	0.697
Erysipelotrichaceae	1.520	1.357	11.322	14.809	2.249	2.310	9.075	13.829
Eubacteriaceae	0.001	0.003	0.000	0.000	0.002	0.006	0.001	0.003
Flavobacteriaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Fusobacteriaceae	0.052	0.161	1.197	3.786	2.361	6.212	0.369	1.167
Gemellaceae	0.005	0.010	0.002	0.004	0.002	0.004	0.002	0.004
Helicobacteraceae	0.000	0.000	0.003	0.010	0.003	0.010	0.000	0.000
Lachnospiraceae	16.233	6.764	11.855	4.918	9.234	3.781	11.869	5.728
Lactobacillaceae	5.070	5.064	5.616	6.792	5.857	5.219	5.463	7.667
Leuconostocaceae	0.011	0.026	0.024	0.047	0.032	0.067	0.025	0.053
Methanobacteriaceae	0.014	0.036	0.005	0.016	0.004	0.013	0.048	0.084
Micrococcaceae	0.007	0.009	0.004	0.010	0.002	0.004	0.006	0.020
Moraxellaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Oxalobacteraceae	0.001	0.003	0.000	0.000	0.001	0.003	0.001	0.003
Pasteurellaceae	0.052	0.129	0.038	0.109	0.016	0.028	0.006	0.016
Peptococcaceae	0.050	0.158	0.025	0.072	0.002	0.007	0.102	0.225
Peptostreptococcaceae	0.013	0.022	0.020	0.030	0.012	0.022	0.009	0.025
Phyllobacteriaceae	0.001	0.003	0.000	0.000	0.004	0.007	0.003	0.007
Planococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Porphyromonadaceae	1.497	1.653	0.886	0.924	0.878	0.758	1.309	1.207

Prevotellaceae	5.672	11.893	2.247	6.169	3.190	7.123	0.538	1.190
Propionibacteriaceae	0.001	0.003	0.001	0.003	0.002	0.006	0.000	0.000
Pseudomonadaceae	0.002	0.007	0.000	0.000	0.001	0.003	0.000	0.000
Rikenellaceae	1.246	1.445	0.964	1.220	1.095	1.450	1.546	1.706
Ruminococcaceae	15.274	6.361	17.811	11.749	14.967	10.031	17.785	13.979
S24-7	1.394	4.130	1.511	4.750	0.056	0.123	0.052	0.124
Sphingobacteriaceae	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000
Staphylococcaceae	0.001	0.003	0.001	0.003	0.000	0.000	0.001	0.003
Streptococcaceae	15.457	15.762	9.793	10.770	21.337	11.921	11.821	12.143
Succinivibrionaceae	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacteraceae	0.053	0.100	0.036	0.076	0.054	0.107	0.019	0.043
Veillonellaceae	1.885	1.143	1.834	1.173	1.213	0.935	2.844	3.817
Verrucomicrobiaceae	0.042	0.119	0.011	0.021	0.062	0.173	0.048	0.152
Victivallaceae	0.000	0.000	0.005	0.010	0.004	0.007	0.004	0.010

Genus	**Time point**							
	T0		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.146	0.207	2.578	3.993	2.795	4.454	5.730	6.089
[Prevotella]	0.976	2.100	0.823	1.626	0.086	0.247	0.002	0.007
[Ruminococcus]	1.019	0.895	0.857	0.903	0.644	0.519	1.881	4.159
Acidaminococcus	0.178	0.282	0.105	0.179	0.067	0.139	0.023	0.045
Actinobacillus	0.005	0.008	0.005	0.017	0.000	0.000	0.000	0.000
Actinomyces	0.022	0.031	0.026	0.048	0.003	0.006	0.007	0.015
Adlercreutzia	0.042	0.073	0.038	0.068	0.062	0.096	0.032	0.072
Aggregatibacter	0.004	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Akkermansia	0.062	0.080	0.067	0.101	0.106	0.205	0.052	0.148
Anaerococcus	0.000	0.000	0.004	0.008	0.000	0.000	0.003	0.009
Anaerostipes	0.076	0.073	0.054	0.052	0.046	0.057	0.018	0.031
Anaerotruncus	0.006	0.008	0.003	0.006	0.011	0.029	0.000	0.000
Arthrobacter	0.000	0.000	0.002	0.005	0.000	0.000	0.000	0.000
Atopobium	0.003	0.007	0.001	0.004	0.000	0.000	0.000	0.000
Bacteroides	38.93	17.23	35.15	15.48	24.60	15.29	18.87	22.73
Bifidobacterium	5.043	3.999	4.586	5.013	8.678	9.764	7.968	12.82
Bilophila	0.193	0.169	1.454	1.066	2.210	2.373	2.432	1.511
Blautia	4.402	2.351	3.642	1.061	3.152	2.402	1.269	0.995
Bulleidia	0.002	0.006	0.052	0.158	0.117	0.371	0.134	0.404
Butyricimonas	0.070	0.099	0.130	0.166	0.040	0.057	0.010	0.017

Campylobacter	0.005	0.008	0.010	0.016	0.006	0.013	0.018	0.033
Catenibacterium	0.000	0.000	2.744	8.659	5.675	17.94	5.979	18.88
cc_115	0.015	0.035	0.024	0.062	0.034	0.072	0.024	0.052
Christensenella	0.000	0.000	0.002	0.005	0.000	0.000	0.001	0.005
Chryseobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chthoniobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.004
Citrobacter	0.014	0.045	3.116	9.819	0.128	0.364	5.643	14.12
Clostridium	0.069	0.060	0.165	0.216	0.744	1.762	0.328	0.782
Clostridium	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Clostridium	0.000	0.000	0.003	0.006	0.006	0.015	0.005	0.009
Collinsella	0.529	0.416	0.426	0.312	0.690	0.904	0.181	0.256
Coprobacillus	0.007	0.012	0.019	0.024	0.033	0.045	0.057	0.106
Coprococcus	4.035	2.927	1.932	1.310	2.578	2.296	0.647	0.578
Corynebacterium	0.000	0.000	0.001	0.004	0.000	0.000	0.001	0.004
Cryocola	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dehalobacterium	0.001	0.004	0.000	0.000	0.003	0.010	0.000	0.000
Desulfovibrio	0.024	0.063	0.056	0.140	0.137	0.281	0.132	0.275
Dialister	0.108	0.184	0.607	1.098	0.627	0.800	0.290	0.571
Dorea	0.773	0.440	0.669	0.389	1.536	1.983	0.801	1.359
Eggerthella	0.045	0.068	0.028	0.052	0.069	0.110	0.009	0.012
Eikenella	0.001	0.004	0.004	0.009	0.001	0.004	0.000	0.000
Enterobacter	0.000	0.000	0.005	0.011	0.001	0.004	0.010	0.027
Enterococcus	0.895	1.212	0.648	1.254	0.636	1.257	4.146	10.56
Epulopiscium	0.002	0.006	0.002	0.005	0.006	0.010	0.000	0.000
Erwinia	0.002	0.006	0.004	0.009	0.000	0.000	0.002	0.008
Escherichia	0.190	0.369	0.283	0.589	0.254	0.310	0.172	0.363
Facklamia	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Faecalibacterium	3.522	2.394	4.501	2.691	2.702	2.604	0.756	1.101
Finegoldia	0.004	0.008	0.000	0.000	0.002	0.007	0.000	0.000
Flavobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.010
Fusobacterium	0.014	0.018	1.554	4.834	1.456	3.651	2.439	5.145
Gemella	0.006	0.008	0.004	0.009	0.000	0.000	0.000	0.000
Granulicatella	0.025	0.043	0.014	0.013	0.004	0.006	0.006	0.010
Haemophilus	0.277	0.513	0.086	0.256	0.031	0.098	0.004	0.009
Helicobacter	0.019	0.040	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.004	0.009	0.035	0.029	0.027	0.036	0.046	0.046
Janthinobacterium	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000
Kingella	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000

Klebsiella	0.000	0.000	0.131	0.340	0.069	0.212	0.021	0.066
Lachnobacterium	0.186	0.370	0.069	0.186	0.053	0.146	0.044	0.112
Lachnospira	0.721	0.561	0.549	0.417	0.347	0.280	0.413	0.731
Lactobacillus	5.456	7.220	3.326	3.580	4.805	4.758	8.928	11.10
Lactococcus	0.008	0.018	0.021	0.050	0.010	0.025	0.124	0.304
Leptotrichia	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Leuconostoc	0.001	0.004	0.003	0.006	0.009	0.025	0.003	0.010
Megamonas	0.000	0.000	0.048	0.153	0.182	0.576	0.004	0.013
Megasphaera	0.013	0.023	0.319	0.971	1.072	3.346	0.006	0.011
Melissococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.015
Mesorhizobium	0.005	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Methanobrevibacter	0.003	0.011	0.005	0.017	0.009	0.030	0.070	0.216
Mogibacterium	0.000	0.000	0.000	0.000	0.001	0.004	0.000	0.000
Morganella	0.002	0.005	0.034	0.094	0.000	0.000	0.413	1.144
Odoribacter	0.312	0.285	0.272	0.188	0.130	0.160	0.050	0.074
Oscillospira	0.509	0.264	2.157	1.303	2.515	2.483	4.656	4.496
Other	0.001	0.004	0.000	0.000	0.002	0.005	0.000	0.000
p-75-a5	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Parabacteroides	1.212	0.985	1.783	1.727	1.193	1.343	1.651	4.646
Paraprevotella	0.208	0.339	0.172	0.262	0.090	0.177	0.051	0.142
Pediococcus	0.001	0.004	0.001	0.004	0.002	0.005	0.014	0.032
Peptoniphilus	0.000	0.000	0.002	0.005	0.003	0.011	0.164	0.514
Peptostreptococcus	0.000	0.000	0.005	0.015	0.006	0.018	0.033	0.103
Phascolarctobacterium	1.190	1.683	1.553	1.759	1.192	1.312	0.285	0.433
Prevotella	4.432	9.245	6.379	9.560	4.840	10.46	2.554	8.053
Proteus	0.006	0.013	0.000	0.000	0.007	0.022	0.005	0.011
PSB-M-3	0.000	0.000	0.001	0.004	0.003	0.011	0.001	0.004
Pseudomonas	0.001	0.005	0.001	0.005	0.002	0.006	0.000	0.000
Pseudoramibacter_Eubacterium	0.002	0.006	0.000	0.000	0.003	0.011	0.000	0.000
Pyramidobacter	0.001	0.004	0.003	0.009	0.005	0.015	0.014	0.045
Ralstonia	0.002	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Roseburia	0.452	0.450	0.155	0.248	0.234	0.349	0.024	0.040
Rothia	0.015	0.024	0.007	0.014	0.003	0.005	0.005	0.011
Ruminococcus	2.183	1.682	1.734	1.485	1.755	2.210	1.325	1.967
Scardovia	0.000	0.000	0.002	0.008	0.000	0.000	0.002	0.007
Selenomonas	0.004	0.008	0.003	0.008	0.001	0.004	0.006	0.018
Serratia	0.004	0.009	0.031	0.071	0.074	0.212	0.877	2.560
SHD-231	0.000	0.000	0.000	0.000	0.002	0.006	0.000	0.000

Slackia	0.021	0.033	0.015	0.021	0.098	0.136	0.006	0.007
SMB53	0.008	0.014	0.024	0.053	0.006	0.012	0.016	0.037
Staphylococcus	0.007	0.014	0.000	0.000	0.000	0.000	0.000	0.000
Streptococcus	18.90	19.456	11.534	10.999	17.966	14.974	13.441	16.239
Sutterella	1.153	0.600	2.497	2.445	2.835	2.001	4.595	3.971
Tetragenococcus	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacter	0.010	0.018	0.039	0.115	0.021	0.054	0.020	0.050
Veillonella	1.190	2.077	0.604	1.418	0.451	0.952	0.033	0.047

Table A11. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) at the baseline, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	4.520	1.650	2.925	1.943	18.745	24.296	9.805	8.625
Bacteroidetes	25.522	20.101	38.400	3.596	30.312	13.821	34.536	12.352
Cyanobacteria	0.000	0.000	0.013	0.023	0.000	0.000	0.017	0.021
Euryarchaeota	0.000	0.000	0.000	0.000	0.030	0.052	0.000	0.000
Firmicutes	65.358	22.118	48.444	6.419	43.107	16.519	44.818	13.522
Fusobacteria	0.000	0.000	0.010	0.017	0.003	0.006	0.118	0.102
Proteobacteria	4.567	4.967	10.059	10.419	7.733	6.514	10.672	9.953
Tenericutes	0.000	0.000	0.013	0.023	0.000	0.000	0.000	0.000
Verrucomicrobia	0.034	0.041	0.135	0.216	0.071	0.123	0.034	0.050

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Cerasicoccales]	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Actinomycetales	0.013	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Bacillales	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Bacteroidales	25.522	20.101	38.402	3.593	30.312	13.821	34.541	12.358
Bifidobacteriales	3.604	1.325	1.787	1.364	17.476	25.346	8.707	8.890
Burkholderiales	1.992	2.132	1.021	1.260	1.151	0.730	1.482	0.802
Campylobacterales	0.007	0.012	0.000	0.000	0.000	0.000	0.003	0.006
Clostridiales	47.104	5.059	43.314	9.985	37.728	21.446	31.330	10.830
Coriobacteriales	0.903	0.515	1.138	0.587	1.269	1.080	1.098	0.797
CW040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Desulfovibrionales	0.972	0.545	1.277	1.215	1.085	1.159	0.484	0.403
Enterobacteriales	1.562	2.635	7.752	9.218	5.494	5.214	8.635	8.660

Erysipelotrichales	16.133	27.010	1.980	2.351	1.733	1.486	8.001	11.887
Fusobacteriales	0.000	0.000	0.010	0.017	0.003	0.006	0.118	0.102
Gemellales	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
I025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lactobacillales	2.115	2.234	3.114	5.306	3.643	6.214	5.480	3.860
Methanobacteriales	0.000	0.000	0.000	0.000	0.030	0.052	0.000	0.000
Pasteurellales	0.030	0.044	0.000	0.000	0.000	0.000	0.047	0.073
RF32	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.023
RF39	0.000	0.000	0.013	0.023	0.000	0.000	0.000	0.000
Rhizobiales	0.003	0.006	0.003	0.006	0.003	0.006	0.000	0.000
SHA-98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacterales	0.003	0.006	0.040	0.070	0.003	0.006	0.007	0.012
Verrucomicrobiales	0.034	0.041	0.131	0.219	0.071	0.123	0.034	0.050
YS2	0.000	0.000	0.013	0.023	0.000	0.000	0.017	0.021

	Time point							
Family	**T0 mean ± sd**		**T5 mean ± sd**		**T10 mean ± sd**		**T24 mean ± sd**	
[Barnesiellaceae]	0.407	0.525	0.255	0.274	0.255	0.347	0.191	0.268
[Mogibacteriaceae]	0.042	0.038	0.094	0.083	0.131	0.120	0.045	0.051
[Odoribacteraceae]	0.288	0.253	0.386	0.180	0.231	0.118	0.208	0.112
[Paraprevotellaceae]	0.000	0.000	0.816	1.155	0.395	0.683	0.205	0.356
[Tissierellaceae]	0.010	0.010	0.004	0.006	0.017	0.029	0.000	0.000
Actinomycetaceae	0.014	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Aerococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Alcaligenaceae	2.054	2.191	1.035	1.252	1.183	0.748	1.501	0.809
Bacillaceae	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Bacteroidaceae	19.480	15.218	27.666	6.019	21.982	6.817	29.814	13.577
Bifidobacteriaceae	3.732	1.418	1.892	1.437	17.654	25.398	8.884	9.154
Campylobacteraceae	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Carnobacteriaceae	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Christensenellaceae	0.007	0.006	0.033	0.040	0.007	0.012	0.024	0.033
Clostridiaceae	0.453	0.624	0.607	0.305	0.668	0.275	0.333	0.365
Coriobacteriaceae	0.941	0.558	1.207	0.627	1.334	1.142	1.108	0.794
Dehalobacteriaceae	0.000	0.000	0.007	0.012	0.004	0.006	0.000	0.000
Desulfovibrionaceae	0.996	0.544	1.339	1.279	1.116	1.206	0.490	0.407
Enterobacteriaceae	1.607	2.712	7.876	9.232	5.590	5.254	8.756	8.759
Enterococcaceae	1.042	1.020	0.031	0.027	2.003	3.434	0.984	0.865

Erysipelotrichaceae	16.328	27.299	2.156	2.629	1.814	1.597	8.044	11.913
Eubacteriaceae	0.000	0.000	0.007	0.013	0.010	0.018	0.003	0.006
Fusobacteriaceae	0.000	0.000	0.010	0.018	0.003	0.006	0.119	0.103
Helicobacteraceae	0.007	0.012	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	16.673	11.877	21.580	4.245	19.048	12.661	13.474	7.054
Lactobacillaceae	0.180	0.189	0.324	0.552	0.618	1.062	1.291	1.120
Leuconostocaceae	0.000	0.000	0.007	0.012	0.000	0.000	0.031	0.045
Methanobacteriaceae	0.000	0.000	0.000	0.000	0.032	0.055	0.000	0.000
Oxalobacteraceae	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Pasteurellaceae	0.031	0.046	0.000	0.000	0.000	0.000	0.048	0.074
Peptococcaceae	0.000	0.000	0.000	0.000	0.160	0.277	0.000	0.000
Peptostreptococcaceae	0.003	0.006	0.004	0.006	0.003	0.006	0.014	0.012
Phyllobacteriaceae	0.003	0.006	0.004	0.006	0.004	0.006	0.000	0.000
Porphyromonadaceae	1.147	0.914	2.599	0.488	1.582	1.733	2.085	1.571
Prevotellaceae	0.003	0.006	4.046	3.695	1.247	2.152	1.051	1.820
Rikenellaceae	0.958	1.012	1.159	0.979	0.938	0.880	0.887	0.746
Ruminococcaceae	26.820	8.990	15.116	4.046	12.159	10.069	14.728	5.443
S24-7	4.354	7.532	3.653	4.186	4.968	8.606	0.525	0.901
Streptococcaceae	0.937	1.091	2.763	4.739	1.024	1.719	3.262	2.064
Turicibacteraceae	0.004	0.006	0.044	0.077	0.003	0.006	0.007	0.012
Veillonellaceae	1.441	1.244	3.130	0.547	3.740	2.842	1.850	1.982
Verrucomicrobiaceae	0.035	0.043	0.138	0.230	0.074	0.127	0.034	0.050

	Time point							
Genus	**T0**		**T5**		**T0**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.397	0.674	1.906	2.995	1.338	2.252	2.248	3.464
[Prevotella]	0.000	0.000	0.978	1.695	0.177	0.306	0.299	0.519
[Ruminococcus]	1.444	0.439	1.332	1.215	0.988	1.055	1.206	1.580
Acidaminococcus	1.203	1.562	0.109	0.189	2.384	4.129	0.113	0.196
Actinomyces	0.037	0.023	0.000	0.000	0.000	0.000	0.000	0.000
Adlercreutzia	0.220	0.151	0.042	0.073	0.071	0.112	0.045	0.079
Akkermansia	0.072	0.063	0.205	0.343	0.128	0.221	0.045	0.064
Anaerobacillus	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Anaerococcus	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Anaerofustis	0.000	0.000	0.010	0.018	0.000	0.000	0.005	0.008
Anaerostipes	0.092	0.133	0.073	0.056	0.116	0.119	0.041	0.060

Anaerotruncus	0.000	0.000	0.021	0.024	0.012	0.021	0.010	0.009
Bacteroides	33.499	18.319	40.506	6.861	32.176	13.340	41.822	16.138
Bifidobacterium	11.862	10.855	2.785	2.147	22.740	31.674	12.516	11.890
Bilophila	3.579	4.569	1.946	1.829	1.657	1.951	0.670	0.498
Blautia	7.582	2.214	7.865	4.312	6.754	6.564	3.576	3.789
Butyricimonas	0.057	0.055	0.228	0.200	0.150	0.179	0.041	0.036
Catenibacterium	0.000	0.000	0.005	0.009	0.012	0.021	0.005	0.008
cc_115	0.046	0.067	0.212	0.367	0.009	0.008	0.000	0.000
Citrobacter	0.012	0.020	0.021	0.024	0.018	0.032	2.502	4.333
Clostridium	0.100	0.074	0.380	0.563	0.253	0.291	0.073	0.072
Clostridium	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000
Collinsella	1.522	0.696	0.942	0.594	1.408	1.212	1.400	1.595
Coprobacillus	0.108	0.174	0.005	0.009	0.006	0.011	0.009	0.008
Coprococcus	6.777	1.868	4.402	0.826	4.974	4.071	5.857	5.287
Dehalobacterium	0.000	0.000	0.011	0.018	0.005	0.008	0.000	0.000
Desulfovibrio	0.122	0.212	0.042	0.073	0.116	0.169	0.000	0.000
Dialister	0.730	0.686	0.960	1.663	0.637	1.093	0.191	0.331
Dorea	1.604	1.591	3.633	4.824	2.139	2.776	0.426	0.241
Eggerthella	0.000	0.000	0.052	0.065	0.019	0.019	0.122	0.071
Enterococcus	3.065	3.024	0.021	0.036	0.787	1.335	1.230	1.116
Epulopiscium	0.005	0.009	0.000	0.000	0.006	0.011	0.005	0.008
Escherichia	0.104	0.181	0.276	0.363	0.203	0.205	0.180	0.105
Faecalibacterium	6.715	2.696	3.930	1.606	4.162	3.559	0.908	0.645
Finegoldia	0.000	0.000	0.005	0.009	0.013	0.022	0.000	0.000
Fusobacterium	0.000	0.000	0.016	0.027	0.004	0.007	0.176	0.158
Gemella	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.008
Granulicatella	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Haemophilus	0.048	0.068	0.000	0.000	0.000	0.000	0.058	0.087
Helicobacter	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.017	0.030	0.026	0.018	0.031	0.021	0.044	0.015
Klebsiella	0.000	0.000	0.125	0.216	0.000	0.000	0.036	0.034
Lachnobacterium	0.620	0.866	0.026	0.045	0.005	0.008	0.009	0.016
Lachnospira	2.010	2.418	0.519	0.624	0.378	0.573	0.143	0.175
Lactobacillus	0.518	0.497	0.453	0.771	0.563	0.964	1.538	1.333
Lactococcus	0.000	0.000	0.005	0.009	0.005	0.008	0.000	0.000
Leuconostoc	0.000	0.000	0.010	0.018	0.000	0.000	0.005	0.008
Megasphaera	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.010

Mesorhizobium	0.005	0.009	0.005	0.009	0.005	0.008	0.000	0.000
Methanobrevibacter	0.000	0.000	0.000	0.000	0.042	0.073	0.000	0.000
Morganella	0.083	0.096	0.000	0.000	0.004	0.007	0.427	0.739
Odoribacter	0.420	0.319	0.333	0.195	0.188	0.233	0.247	0.164
Oribacterium	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Oscillospira	2.549	2.725	3.739	4.148	2.226	2.026	2.250	0.572
Parabacteroides	1.936	1.204	3.832	0.751	2.258	2.248	3.206	2.878
Paraprevotella	0.000	0.000	0.120	0.207	0.340	0.589	0.045	0.078
Peptococcus	0.000	0.000	0.000	0.000	0.209	0.363	0.000	0.000
Peptoniphilus	0.026	0.032	0.000	0.000	0.009	0.015	0.000	0.000
Phascolarctobacterium	0.620	1.022	3.541	2.517	2.049	1.780	1.868	2.256
Prevotella	0.005	0.009	5.998	5.631	1.633	2.818	1.763	3.054
Ralstonia	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Rikenella	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000
Roseburia	1.068	1.647	0.172	0.096	0.312	0.329	0.072	0.093
Ruminococcus	3.125	1.304	1.996	1.147	2.935	3.577	5.650	3.596
Serratia	0.000	0.000	0.042	0.073	0.006	0.011	0.110	0.190
Slackia	0.039	0.036	0.169	0.279	0.188	0.225	0.033	0.043
SMB53	0.000	0.000	0.052	0.089	0.013	0.022	0.000	0.000
Streptococcus	2.381	2.060	4.252	7.297	1.291	2.173	4.369	2.509
Sutterella	3.324	2.850	1.580	1.941	1.777	1.363	2.091	0.956
Turicibacter	0.006	0.010	0.062	0.107	0.004	0.007	0.009	0.015
Veillonella	0.228	0.200	0.005	0.009	0.043	0.074	0.281	0.487

Table A12. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon lactulose treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	12.135	16.599	6.954	7.576	7.310	5.711	33.891	28.338
Bacteroidetes	38.739	9.405	33.640	3.230	32.786	6.861	14.206	8.985
Cyanobacteria	0.010	0.010	0.000	0.000	0.007	0.006	0.000	0.000
Euryarchaeota	0.000	0.000	0.007	0.012	0.000	0.000	0.000	0.000
Firmicutes	45.718	7.076	53.231	6.351	56.166	4.160	42.005	20.394
Fusobacteria	0.013	0.012	0.000	0.000	0.003	0.006	0.003	0.006
Lentisphaerae	0.000	0.000	0.007	0.012	0.003	0.006	0.003	0.006
Proteobacteria	3.880	2.636	6.145	9.721	3.721	2.756	9.858	8.284

Tenericutes	0.003	0.006	0.013	0.023	0.003	0.006	0.000	0.000
Verrucomicrobia	0.024	0.015	0.003	0.006	0.000	0.000	0.034	0.029

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Cerasicoccales]	0.003	0.006	0.003	0.006	0.000	0.000	0.000	0.000
Actinomycetales	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Bacteroidales	38.561	9.498	33.640	3.230	32.788	6.862	14.206	8.985
Bifidobacteriales	1.701	0.832	5.986	7.660	6.041	5.991	31.658	28.338
Burkholderiales	0.487	0.294	1.097	1.271	1.177	0.674	2.217	2.558
Campylobacterales	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Clostridiales	29.369	1.886	46.416	8.634	43.876	8.935	29.853	23.791
Coriobacteriales	10.348	16.929	0.968	0.098	1.269	1.290	2.229	3.252
Desulfovibrionales	0.319	0.088	0.943	1.463	0.221	0.156	2.565	4.304
Enterobacteriales	2.988	2.836	4.101	7.016	2.312	1.959	5.073	7.764
Erysipelotrichales	7.826	9.058	5.037	5.484	4.141	4.281	3.498	2.966
Fusobacteriales	0.013	0.012	0.000	0.000	0.003	0.006	0.003	0.006
Gemellales	0.000	0.000	0.000	0.000	0.014	0.023	0.003	0.006
Lactobacillales	8.284	12.007	1.708	2.933	8.137	12.763	8.644	7.616
Methanobacteriales	0.000	0.000	0.007	0.012	0.000	0.000	0.000	0.000
RF32	0.030	0.020	0.003	0.006	0.010	0.018	0.000	0.000
RF39	0.003	0.006	0.013	0.023	0.003	0.006	0.000	0.000
Rhizobiales	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacterales	0.010	0.017	0.070	0.082	0.000	0.000	0.007	0.006
Verrucomicrobiales	0.020	0.020	0.000	0.000	0.000	0.000	0.034	0.029
Victivallales	0.000	0.000	0.007	0.012	0.003	0.006	0.003	0.006
YS2	0.010	0.010	0.000	0.000	0.003	0.006	0.000	0.000

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0,3794	0,1279	0,7490	0,510	0,0598	0,0539	0,2681	0,4644
[Cerasicoccaceae]	0,0035	0,0060	0,0037	0,006	0,0000	0,0000	0,0000	0,0000
[Mogibacteriaceae]	0,0347	0,0423	0,2573	0,059	0,0485	0,0659	0,0068	0,0118
[Odoribacteraceae]	0,3164	0,2939	0,3761	0,104	0,2918	0,0613	0,1409	0,1673
[Paraprevotellaceae]	0,0070	0,0121	0,2853	0,322	1,3218	2,2709	0,0000	0,0000
[Tissierellaceae]	0,0035	0,0060	0,0073	0,006	0,0071	0,0123	0,5192	0,8906
Actinomycetaceae	0,0000	0,0000	0,0000	0,000	0,0000	0,0000	0,0036	0,0062

Alcaligenaceae	0,5015	0,3066	1,1656	1,366	1,2262	0,7155	2,2333	2,5779
Bacteroidaceae	36,640	9,5706	27,115	2,466	20,561	4,3966	12,216	7,1947
Bifidobacteriaceae	1,7544	0,8710	6,1556	7,742	6,2762	6,1973	32,4419	28,4027
Carnobacteriaceae	0,0035	0,0060	0,0036	0,006	0,0137	0,0238	0,0068	0,0118
Christensenellaceae	0,0070	0,0060	0,0765	0,072	0,0834	0,1097	0,0068	0,0118
Clostridiaceae	0,2501	0,1841	0,5574	0,251	0,1912	0,1880	0,2914	0,3775
Coriobacteriaceae	10,490	17,140	1,0316	0,143	1,3318	1,3757	2,3708	3,4897
Dehalobacteriaceae	0,0000	0,0000	0,0036	0,006	0,0000	0,0000	0,0000	0,0000
Desulfovibrionaceae	0,3276	0,0899	1,0118	1,565	0,2317	0,1669	2,5987	4,3539
Enterobacteriaceae	3,0763	2,9431	4,3929	7,517	2,4225	2,0631	5,0856	7,7679
Enterococcaceae	0,2003	0,3199	0,0036	0,006	0,3981	0,6714	3,0137	3,6573
Erysipelotrichaceae	8,0738	9,4085	5,2349	5,546	4,3500	4,5589	3,6623	3,2285
Eubacteriaceae	0,0034	0,0059	0,0000	0,000	0,0036	0,0062	0,0000	0,0000
Fusobacteriaceae	0,0138	0,0119	0,0000	0,000	0,0034	0,0059	0,0034	0,0058
Gemellaceae	0,0000	0,0000	0,0000	0,000	0,0137	0,0238	0,0034	0,0059
Helicobacteraceae	0,0000	0,0000	0,0000	0,000	0,0000	0,0000	0,0034	0,0059
Lachnospiraceae	15,132	4,6100	20,257	7,052	26,899	7,7294	11,559	11,658
Lactobacillaceae	1,7518	2,3337	0,2661	0,451	0,9887	1,6943	0,9608	0,9335
Leptotrichiaceae	0,0000	0,0000	0,0000	0,000	0,0000	0,0000	0,0000	0,0000
Leuconostocaceae	0,0311	0,0314	0,0000	0,000	0,0069	0,0120	0,0000	0,0000
Methanobacteriaceae	0,0000	0,0000	0,0068	0,011	0,0000	0,0000	0,0000	0,0000
Oxalobacteraceae	0,0000	0,0000	0,0000	0,000	0,0036	0,0062	0,0000	0,0000
Pasteurellaceae	0,0243	0,0337	0,0000	0,000	0,0000	0,0000	0,0000	0,0000
Peptococcaceae	0,0000	0,0000	0,1678	0,272	0,0000	0,0000	0,0000	0,0000
Peptostreptococcaceae	0,0452	0,0783	0,0145	0,016	0,0036	0,0062	0,0000	0,0000
Phyllobacteriaceae	0,0035	0,0060	0,0000	0,000	0,0000	0,0000	0,0000	0,0000
Porphyromonadaceae	1,0973	1,2591	2,7411	0,920	2,1340	0,2874	0,4994	0,4637
Prevotellaceae	0,8176	1,4071	2,1047	2,154	6,0392	8,0955	0,0108	0,0187
Rikenellaceae	0,3755	0,5261	1,8692	1,294	1,3105	0,5568	0,6480	0,9676
Ruminococcaceae	10,1368	4,8165	19,6850	1,324	12,792	1,7240	14,541	17,047
S24-7	0,1009	0,1748	0,4838	0,811	2,1078	3,2152	0,8693	1,5058
Streptococcaceae	6,5758	10,2467	1,5570	2,6777	6,8570	11,5589	4,6983	4,3347
Turicibacteraceae	0,0104	0,0181	0,0767	0,0903	0,0000	0,0000	0,0068	0,0058
Veillonellaceae	1,7885	0,5864	2,3319	0,9650	2,0175	0,5591	1,2904	0,4668
Verrucomicrobiaceae	0,0207	0,0209	0,0000	0,0000	0,0000	0,0000	0,0349	0,0303
Victivallaceae	0,0000	0,0000	0,0068	0,0118	0,0035	0,0060	0,0034	0,0059

Genus	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
[Eubacterium]	0.042	0.074	4.884	5.083	0.621	0.313	0.042	0.074
[Prevotella]	0.000	0.000	0.089	0.154	1.451	2.513	0.000	0.000
[Ruminococcus]	0.477	0.406	1.161	1.028	1.797	2.971	0.477	0.406
Acidaminococcus	0.054	0.048	0.369	0.639	0.024	0.025	0.054	0.048
Actinomyces	0.005	0.009	0.000	0.000	0.000	0.000	0.005	0.009
Adlercreutzia	0.063	0.064	0.074	0.128	0.030	0.042	0.063	0.064
Akkermansia	0.049	0.043	0.000	0.000	0.000	0.000	0.049	0.043
Alistipes	0.000	0.000	0.000	0.000	0.007	0.013	0.000	0.000
Anaerococcus	0.000	0.000	0.000	0.000	0.007	0.013	0.000	0.000
Anaerofustis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Anaerostipes	0.056	0.073	0.058	0.055	0.062	0.035	0.056	0.073
Anaerotruncus	0.000	0.000	0.034	0.059	0.004	0.008	0.000	0.000
Bacteroides	16.965	10.303	38.386	6.330	32.238	16.332	16.965	10.303
Bifidobacterium	43.329	36.488	7.955	9.326	9.007	7.473	43.329	36.488
Bilophila	3.575	6.090	1.149	1.726	0.402	0.395	3.575	6.090
Blautia	3.452	3.390	8.917	6.118	9.954	10.878	3.452	3.390
Butyricimonas	0.014	0.025	0.205	0.138	0.171	0.044	0.014	0.025
cc_115	0.023	0.030	0.188	0.325	0.015	0.026	0.023	0.030
Christensenella	0.005	0.008	0.000	0.000	0.000	0.000	0.005	0.008
Clostridium	0.227	0.317	0.121	0.103	0.021	0.036	0.227	0.317
Collinsella	3.160	5.038	0.695	0.302	2.165	3.193	3.160	5.038
Coprobacillus	0.004	0.007	0.000	0.000	0.004	0.007	0.004	0.007
Coprococcus	2.680	3.411	5.203	1.630	6.004	3.719	2.680	3.411
Desulfovibrio	0.030	0.041	0.397	0.688	0.000	0.000	0.030	0.041
Dialister	0.274	0.474	1.095	1.261	0.815	0.811	0.274	0.474
Dorea	0.195	0.258	2.088	1.387	0.772	0.683	0.195	0.258
Eggerthella	0.052	0.089	0.034	0.045	0.016	0.014	0.052	0.089
Enterococcus	1.774	1.523	0.006	0.010	0.491	0.851	1.774	1.523
Escherichia	0.185	0.321	0.174	0.291	0.175	0.178	0.185	0.321
Faecalibacterium	2.751	2.467	4.461	2.603	2.580	1.842	2.751	2.467
Finegoldia	0.004	0.007	0.000	0.000	0.007	0.013	0.004	0.007
Fusobacterium	0.004	0.007	0.000	0.000	0.004	0.007	0.004	0.007
Gemella	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000
Granulicatella	0.009	0.016	0.000	0.000	0.017	0.029	0.009	0.016
Helicobacter	0.005	0.008	0.000	0.000	0.000	0.000	0.005	0.008

Holdemania	0.039	0.023	0.004	0.007	0.004	0.008	0.039	0.023
Lachnobacterium	0.024	0.041	0.037	0.063	0.026	0.045	0.024	0.041
Lachnospira	0.536	0.486	0.798	1.062	0.165	0.083	0.536	0.486
Lactobacillus	1.031	0.942	0.278	0.482	1.128	1.923	1.031	0.942
Lactococcus	0.000	0.000	0.000	0.000	0.017	0.029	0.000	0.000
Megasphaera	0.028	0.049	0.000	0.000	0.004	0.008	0.028	0.049
Methanobrevibacter	0.000	0.000	0.008	0.015	0.000	0.000	0.000	0.000
Morganella	0.019	0.033	0.000	0.000	0.000	0.000	0.019	0.033
Odoribacter	0.183	0.209	0.336	0.243	0.252	0.032	0.183	0.209
Oribacterium	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000
Oscillospira	5.479	9.093	2.530	2.454	1.300	0.729	5.479	9.093
Parabacteroides	0.701	0.647	3.770	0.792	3.327	1.568	0.701	0.647
Paraprevotella	0.000	0.000	0.173	0.300	0.132	0.229	0.000	0.000
Parvimonas	0.000	0.000	0.006	0.010	0.000	0.000	0.000	0.000
Peptococcus	0.000	0.000	0.199	0.344	0.000	0.000	0.000	0.000
Peptoniphilus	0.703	1.218	0.005	0.009	0.000	0.000	0.703	1.218
ph2	0.005	0.008	0.000	0.000	0.000	0.000	0.005	0.008
Phascolarctobacterium	1.324	1.150	1.930	2.021	2.054	0.916	1.324	1.150
Prevotella	0.015	0.027	2.870	3.079	8.115	9.356	0.015	0.027
Proteus	0.009	0.008	0.000	0.000	0.000	0.000	0.009	0.008
Roseburia	0.136	0.176	0.107	0.071	0.183	0.147	0.136	0.176
Ruminococcus	1.555	2.294	4.800	1.909	3.992	1.848	1.555	2.294
Serratia	0.071	0.123	0.000	0.000	0.000	0.000	0.071	0.123
Slackia	0.005	0.008	0.016	0.017	0.064	0.065	0.005	0.008
SMB53	0.004	0.007	0.075	0.071	0.000	0.000	0.004	0.007
Streptococcus	6.327	5.973	2.457	4.228	8.286	13.854	6.327	5.973
Sutterella	2.251	2.286	1.735	2.228	2.043	1.699	2.251	2.286
Turicibacter	0.009	0.008	0.112	0.127	0.000	0.000	0.009	0.008
Veillonella	0.071	0.066	0.000	0.000	0.012	0.011	0.071	0.066

Table A13. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon lactulose + rifaximin treatment, over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	1.573	1.093	2.737	1.423	1.302	0.195	2.367	1.487
Bacteroidetes	39.376	15.275	34.750	4.497	30.786	28.082	32.603	4.689

Cyanobacteria	0.017	0.015	0.000	0.000	0.003	0.006	0.000	0.000
Euryarchaeota	0.000	0.000	0.000	0.000	0.003	0.006	0.253	0.358
Firmicutes	44.601	10.202	55.352	6.925	58.763	17.964	62.871	5.754
Fusobacteria	0.121	0.210	0.000	0.000	0.003	0.006	0.000	0.000
Lentisphaerae	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Proteobacteria	14.292	11.116	7.097	9.466	9.119	11.852	1.891	0.086
Tenericutes	0.007	0.006	0.023	0.041	0.000	0.000	0.000	0.000
Verrucomicrobia	0.013	0.015	0.041	0.037	0.017	0.029	0.015	0.021

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinomycetales	0.013	0.015	0.013	0.015	0.010	0.017	0.000	0.000
Bacteroidales	39.379	15.275	34.750	4.497	30.786	28.082	32.603	4.689
Bifidobacteriales	0.664	0.776	1.731	1.563	0.714	0.417	1.891	1.258
Burkholderiales	2.093	1.388	0.598	0.904	2.297	2.763	1.517	0.258
Campylobacterales	0.003	0.006	0.013	0.023	0.007	0.012	0.000	0.000
Clostridiales	37.518	3.693	38.300	18.762	23.079	11.040	31.851	6.460
Coriobacteriales	0.896	0.326	0.993	0.319	0.578	0.246	0.475	0.229
Desulfovibrionales	0.800	1.055	1.242	1.003	1.139	0.839	0.238	0.336
Enterobacteriales	11.336	9.534	5.244	9.004	5.616	8.705	0.137	0.165
Erysipelotrichales	0.537	0.209	12.271	16.205	34.132	29.222	31.010	0.706
Fusobacteriales	0.121	0.210	0.000	0.000	0.003	0.006	0.000	0.000
Gemellales	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Lactobacillales	6.545	6.754	4.721	8.124	1.537	2.644	0.010	0.000
Methanobacteriales	0.000	0.000	0.000	0.000	0.003	0.006	0.253	0.358
Pasteurellales	0.020	0.027	0.000	0.000	0.023	0.041	0.000	0.000
Pseudomonadales	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RF32	0.030	0.030	0.000	0.000	0.020	0.035	0.000	0.000
RF39	0.007	0.006	0.023	0.041	0.000	0.000	0.000	0.000
Rhizobiales	0.003	0.006	0.000	0.000	0.003	0.006	0.000	0.000
Turicibacterales	0.003	0.006	0.060	0.105	0.024	0.025	0.000	0.000
Verrucomicrobiales	0.013	0.015	0.041	0.037	0.017	0.029	0.015	0.021
Victivallales	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
YS2	0.017	0.015	0.000	0.000	0.003	0.006	0.000	0.000

Family	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
[Barnesiellaceae]	0.314	0.414	0.558	0.161	0.351	0.497	0.021	0.029
[Mogibacteriaceae]	0.383	0.483	0.196	0.146	0.037	0.052	0.127	0.180
[Odoribacteraceae]	0.456	0.125	0.537	0.073	0.251	0.327	0.158	0.006
[Paraprevotellaceae]	0.000	0.000	0.067	0.094	0.000	0.000	1.643	2.324
[Tissierellaceae]	0.000	0.000	0.005	0.007	0.000	0.000	0.000	0.000
[Weeksellaceae]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Actinomycetaceae	0.021	0.015	0.011	0.001	0.016	0.022	0.000	0.000
Alcaligenaceae	1.348	0.085	0.887	1.128	0.757	0.768	1.527	0.258
Bacteroidaceae	37.099	25.025	29.444	1.704	45.500	17.433	26.887	5.831
Bifidobacteriaceae	0.945	0.966	2.297	1.881	0.802	0.556	1.915	1.283
Campylobacteraceae	0.005	0.007	0.000	0.000	0.010	0.015	0.000	0.000
Carnobacteriaceae	0.000	0.000	0.005	0.007	0.010	0.015	0.000	0.000
Christensenellaceae	0.173	0.230	0.105	0.119	0.016	0.008	0.010	0.000
Clostridiaceae	0.588	0.268	0.547	0.672	0.245	0.246	0.332	0.106
Coriobacteriaceae	1.002	0.455	1.148	0.498	0.598	0.370	0.481	0.234
Corynebacteriaceae	0.000	0.000	0.005	0.007	0.000	0.000	0.000	0.000
Dehalobacteriaceae	0.016	0.022	0.000	0.000	0.000	0.000	0.000	0.000
Desulfovibrionaceae	0.199	0.015	0.783	0.778	0.891	1.008	0.241	0.341
Dethiosulfovibrionaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Enterobacteriaceae	8.530	11.515	0.048	0.005	0.604	0.099	0.137	0.165
Enterococcaceae	0.452	0.550	0.982	1.388	0.026	0.037	0.000	0.000
Erysipelotrichaceae	0.435	0.036	3.325	3.986	25.508	35.481	31.333	0.908
Eubacteriaceae	0.005	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacteriaceae	0.000	0.000	0.000	0.000	0.005	0.007	0.000	0.000
Gemellaceae	0.000	0.000	0.000	0.000	0.005	0.007	0.000	0.000
Helicobacteraceae	0.000	0.000	0.021	0.029	0.000	0.000	0.000	0.000
Lachnospiraceae	18.321	2.763	15.981	8.003	10.483	8.946	17.791	11.824
Lactobacillaceae	2.091	0.669	0.852	1.206	1.190	1.683	0.000	0.000
Leuconostocaceae	0.126	0.044	0.000	0.000	0.079	0.111	0.000	0.000
Methanobacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.254	0.360
Microbacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Micrococcaceae	0.000	0.000	0.005	0.007	0.000	0.000	0.000	0.000
Oxalobacteraceae	0.000	0.000	0.006	0.008	0.000	0.000	0.005	0.007
Pasteurellaceae	0.026	0.037	0.000	0.000	0.037	0.052	0.000	0.000
Peptococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.101

Peptostreptococcaceae	0.010	0.000	0.022	0.031	0.005	0.007	0.000	0.000
Phyllobacteriaceae	0.005	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Porphyromonadaceae	1.175	0.745	1.809	1.448	0.313	0.298	0.532	0.307
Prevotellaceae	0.011	0.015	2.499	3.520	0.000	0.000	1.369	1.921
Rikenellaceae	1.807	2.407	2.228	0.023	0.115	0.105	0.271	0.096
Ruminococcaceae	15.615	5.312	27.453	9.368	9.448	3.395	11.747	5.446
S24-7	0.000	0.000	0.961	1.359	0.296	0.419	2.068	2.924
Streptococcaceae	7.019	7.040	5.416	7.596	1.090	1.528	0.010	0.000
Succinivibrionaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacteraceae	0.005	0.007	0.100	0.141	0.010	0.015	0.000	0.000
Veillonellaceae	1.802	0.959	1.673	1.781	1.272	1.582	1.052	0.182
Verrucomicrobiaceae	0.016	0.022	0.026	0.037	0.026	0.037	0.015	0.022

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.152	0.110	2.557	3.535	1.665	2.853	7.946	11.238
[Prevotella]	1.158	2.006	0.000	0.000	0.000	0.000	0.000	0.000
[Ruminococcus]	0.905	0.666	0.780	0.581	0.758	0.330	0.597	0.438
Acidaminococcus	0.810	1.403	0.072	0.126	0.000	0.000	0.000	0.000
Actinobacillus	0.000	0.000	0.000	0.000	0.008	0.014	0.000	0.000
Actinomyces	0.018	0.019	0.005	0.009	0.013	0.022	0.000	0.000
Adlercreutzia	0.077	0.133	0.084	0.117	0.016	0.028	0.010	0.015
Akkermansia	0.018	0.019	0.067	0.063	0.021	0.036	0.031	0.044
Anaerofustis	0.006	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Anaerostipes	0.092	0.067	0.032	0.032	0.092	0.159	0.045	0.005
Anaerotruncus	0.027	0.025	0.000	0.000	0.000	0.000	0.000	0.000
Bacteroides	44.679	18.885	47.383	7.263	51.351	39.010	48.983	18.792
Bifidobacterium	1.051	1.307	2.733	2.354	2.181	1.480	3.623	2.890
Bilophila	1.211	1.616	2.047	1.735	4.455	4.669	0.381	0.539
Blautia	6.583	2.051	5.395	5.562	3.176	2.425	6.707	1.181
Bulleidia	0.004	0.007	0.000	0.000	0.077	0.133	0.000	0.000
Butyricimonas	0.177	0.204	0.227	0.293	0.000	0.000	0.120	0.169
Campylobacter	0.004	0.007	0.000	0.000	0.008	0.014	0.000	0.000
Catenibacterium	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
cc_115	0.000	0.000	0.233	0.326	0.000	0.000	0.000	0.000
Citrobacter	0.025	0.044	2.990	5.178	0.156	0.031	0.191	0.271
Clostridium	0.233	0.225	0.032	0.014	0.066	0.095	0.074	0.076

Collinsella	0.464	0.331	0.877	0.267	0.952	0.697	0.572	0.357
Coprobacillus	0.011	0.009	0.010	0.018	0.235	0.396	0.000	0.000
Coprococcus	4.110	1.954	4.014	2.077	1.370	0.646	11.585	14.081
Corynebacterium	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Dehalobacterium	0.017	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Desulfovibrio	0.000	0.000	0.012	0.020	0.032	0.056	0.072	0.102
Dialister	0.380	0.658	1.304	1.983	2.518	4.362	0.854	1.207
Dorea	1.140	0.753	0.564	0.307	0.620	0.482	0.873	0.390
Eggerthella	0.148	0.179	0.011	0.018	0.029	0.051	0.000	0.000
Enterococcus	0.483	0.726	0.958	1.659	0.017	0.029	0.000	0.000
Escherichia	0.590	0.511	0.005	0.009	1.000	1.731	0.000	0.000
Faecalibacterium	2.531	1.661	3.405	2.628	1.672	1.355	4.809	6.779
Finegoldia	0.000	0.000	0.000	0.000	0.019	0.033	0.000	0.000
Fusobacterium	0.185	0.320	0.000	0.000	0.004	0.007	0.000	0.000
Gemella	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000
Granulicatella	0.015	0.027	0.000	0.000	0.008	0.014	0.000	0.000
Haemophilus	0.026	0.033	0.000	0.000	0.021	0.036	0.000	0.000
Helicobacter	0.000	0.000	0.010	0.018	0.000	0.000	0.000	0.000
Holdemania	0.091	0.091	0.069	0.079	0.072	0.067	0.018	0.003
Klebsiella	0.072	0.124	0.000	0.000	0.000	0.000	0.000	0.000
Lachnobacterium	0.011	0.019	0.213	0.256	0.000	0.000	0.000	0.000
Lachnospira	0.867	1.027	0.734	0.514	1.019	0.684	0.824	1.166
Lactobacillus	1.763	1.592	0.782	1.340	0.947	1.640	0.000	0.000
Lactococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.011
Leuconostoc	0.050	0.086	0.000	0.000	0.004	0.007	0.000	0.000
Megamonas	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Megasphaera	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000
Mesorhizobium	0.006	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Methanobrevibacter	0.000	0.000	0.000	0.000	0.019	0.033	0.399	0.564
Morganella	0.000	0.000	0.005	0.009	0.004	0.007	0.000	0.000
Odoribacter	0.460	0.190	0.309	0.286	0.246	0.287	0.163	0.207
Oribacterium	0.004	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Oscillospira	3.869	3.132	2.704	0.958	8.943	13.234	1.694	0.560
p-75-a5	0.000	0.000	0.000	0.000	0.346	0.599	0.000	0.000
Parabacteroides	3.039	2.458	1.819	2.004	0.366	0.225	0.999	0.714
Paraprevotella	0.113	0.195	0.000	0.000	0.000	0.000	2.577	3.644
Peptococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.112	0.158
Peptoniphilus	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000

Phascolarctobacterium	3.068	2.721	1.687	2.404	1.064	1.280	0.981	1.207
Prevotella	3.409	5.877	2.391	4.128	0.038	0.067	2.148	3.009
Ralstonia	0.000	0.000	0.005	0.009	0.019	0.033	0.010	0.015
Roseburia	0.433	0.309	0.110	0.096	0.200	0.326	0.096	0.068
Rothia	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Ruminococcus	4.388	2.739	2.746	2.277	1.567	1.626	0.776	0.573
Serratia	0.013	0.022	4.623	8.007	0.391	0.644	0.000	0.000
Slackia	0.213	0.317	0.071	0.064	0.000	0.000	0.018	0.003
SMB53	0.008	0.015	0.021	0.037	0.062	0.096	0.000	0.000
Streptococcus	7.536	9.819	5.421	9.334	0.891	1.472	0.010	0.015
Sutterella	3.126	2.191	0.332	0.372	11.009	17.293	1.685	1.435
Turicibacter	0.004	0.007	0.096	0.166	0.104	0.160	0.000	0.000
Varibaculum	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Veillonella	0.123	0.200	0.026	0.045	0.100	0.173	0.008	0.011

Table A14. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon rifaximin treatment, over time.

	Time point							
Phylum	**T0 mean ± sd**		**T5 mean ± sd**		**T10 mean ± sd**		**T24 mean ± sd**	
Actinobacteria	10.870	11.999	4.543	2.015	4.875	1.362	3.294	2.957
Bacteroidetes	33.474	2.480	35.162	9.343	32.021	9.560	34.833	9.869
Cyanobacteria	0.174	0.199	0.003	0.006	0.003	0.006	0.007	0.012
Euryarchaeota	0.610	1.056	0.121	0.132	0.024	0.041	0.000	0.000
Firmicutes	51.749	11.493	55.019	15.698	52.510	10.243	41.104	6.062
Fusobacteria	0.000	0.000	0.000	0.000	0.000	0.000	8.555	14.818
Lentisphaerae	0.044	0.075	0.003	0.006	0.000	0.000	0.000	0.000
Proteobacteria	3.814	3.630	4.469	6.365	10.536	9.206	12.176	8.172
Synergistetes	0.000	0.000	0.239	0.413	0.000	0.000	0.000	0.000
Tenericutes	0.000	0.000	0.003	0.006	0.000	0.000	0.003	0.006
Verrucomicrobia	0.000	0.000	0.437	0.757	0.030	0.044	0.027	0.025

	Time point							
Order	**T0 mean ± sd**		**T5 mean ± sd**		**T10 mean ± sd**		**T24 mean ± sd**	
[Cerasicoccales]	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Actinomycetales	0.007	0.012	0.010	0.010	0.013	0.006	0.013	0.012
Bacillales	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000

Bacteroidales	25.751	19.086	35.163	9.344	32.023	9.561	34.833	9.869
Bifidobacteriales	7.618	9.730	3.486	1.823	3.549	0.293	2.500	2.509
Burkholderiales	1.525	1.151	0.809	0.348	3.133	2.498	2.720	2.742
Campylobacterales	0.000	0.000	0.000	0.000	0.010	0.017	0.000	0.000
Clostridiales	42.357	25.222	45.726	5.212	48.319	6.889	32.170	13.179
Coriobacteriales	0.744	0.753	1.047	0.718	1.313	1.108	0.781	0.448
Desulfovibrionales	0.458	0.404	1.391	2.306	3.822	3.317	4.294	2.240
Enterobacteriales	0.945	1.506	2.252	3.718	3.560	4.808	5.156	8.720
Erysipelotrichales	9.773	13.481	1.353	1.178	1.911	1.563	3.554	3.899
Fusobacteriales	0.000	0.000	0.000	0.000	0.000	0.000	8.555	14.818
Lactobacillales	10.672	17.466	7.919	13.472	2.282	3.848	5.282	9.079
Methanobacteriales	0.047	0.081	0.121	0.132	0.024	0.041	0.000	0.000
ML615J-28	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Pseudomonadales	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
RF32	0.000	0.000	0.013	0.023	0.000	0.000	0.003	0.006
RF39	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Rhizobiales	0.003	0.006	0.000	0.000	0.007	0.006	0.003	0.006
SHA-98	0.000	0.000	0.007	0.012	0.000	0.000	0.000	0.000
Synergistales	0.000	0.000	0.239	0.413	0.000	0.000	0.000	0.000
Turicibacterales	0.084	0.051	0.013	0.023	0.000	0.000	0.098	0.160
Verrucomicrobiales	0.000	0.000	0.434	0.751	0.030	0.044	0.027	0.025
Victivallales	0.003	0.006	0.003	0.006	0.000	0.000	0.000	0.000
YS2	0.010	0.017	0.003	0.006	0.000	0.000	0.007	0.012

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.681	0.697	0.565	0.478	0.447	0.369	0.193	0.196
[Cerasicoccaceae]	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
[Mogibacteriaceae]	0.272	0.332	0.204	0.141	0.110	0.137	0.032	0.028
[Odoribacteraceae]	0.411	0.358	0.609	0.145	0.421	0.190	0.371	0.189
[Paraprevotellaceae]	0.253	0.438	0.838	0.800	0.417	0.723	0.220	0.371
[Tissierellaceae]	0.000	0.000	0.000	0.000	0.007	0.006	0.004	0.006
Actinomycetaceae	0.003	0.006	0.010	0.011	0.010	0.011	0.007	0.012
Alcaligenaceae	1.575	1.124	0.827	0.353	3.222	2.549	2.765	2.743
Bacteroidaceae	21.764	15.356	28.000	6.523	25.051	6.558	26.925	3.668
Bifidobacteriaceae	7.760	9.725	3.605	1.825	3.707	0.380	2.616	2.638
Burkholderiaceae	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.000

Carnobacteriaceae	0.015	0.025	0.000	0.000	0.007	0.012	0.007	0.012
Christensenellaceae	0.022	0.029	0.207	0.277	0.036	0.062	0.039	0.059
Clostridiaceae	2.796	2.259	0.381	0.392	0.542	0.553	0.482	0.283
Comamonadaceae	0.000	0.000	0.004	0.006	0.003	0.006	0.000	0.000
Coriobacteriaceae	0.795	0.802	1.091	0.758	1.381	1.186	0.817	0.476
Corynebacteriaceae	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000
Desulfovibrionaceae	0.482	0.432	1.421	2.352	3.956	3.439	4.445	2.270
Dethiosulfovibrionaceae	0.000	0.000	0.243	0.422	0.000	0.000	0.000	0.000
Enterobacteriaceae	0.955	1.514	2.299	3.792	3.719	5.055	5.503	9.315
Enterococcaceae	0.024	0.026	0.063	0.110	1.056	1.819	0.014	0.012
Erysipelotrichaceae	9.925	13.512	1.411	1.243	2.008	1.674	3.758	4.196
Eubacteriaceae	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Fusobacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	8.584	14.868
Helicobacteraceae	0.000	0.000	0.000	0.000	0.010	0.018	0.000	0.000
Lachnospiraceae	16.348	13.348	15.980	8.158	16.078	5.442	9.833	5.278
Lactobacillaceae	2.104	3.568	1.274	2.032	0.130	0.216	0.409	0.699
Leuconostocaceae	0.000	0.000	0.011	0.018	0.000	0.000	0.007	0.012
Methanobacteriaceae	0.050	0.086	0.124	0.135	0.025	0.043	0.000	0.000
Microbacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Micrococcaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.012
Moraxellaceae	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000
Oxalobacteraceae	0.000	0.000	0.004	0.006	0.007	0.012	0.004	0.006
Pasteurellaceae	0.004	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Peptococcaceae	0.150	0.232	0.113	0.195	0.189	0.328	0.000	0.000
Peptostreptococcaceae	0.018	0.032	0.007	0.006	0.004	0.006	0.004	0.006
Phyllobacteriaceae	0.004	0.006	0.000	0.000	0.007	0.006	0.004	0.006
Porphyromonadaceae	1.684	1.764	2.679	1.393	2.111	1.856	2.026	0.602
Prevotellaceae	1.318	2.275	1.266	2.165	1.680	2.901	0.339	0.502
Propionibacteriaceae	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Rikenellaceae	1.483	0.985	2.462	0.780	1.667	1.094	0.984	0.617
Ruminococcaceae	18.760	12.961	24.669	2.963	27.820	9.429	17.392	9.364
S24-7	0.000	0.000	0.113	0.187	1.750	3.031	5.191	7.723
Streptococcaceae	8.630	13.972	6.855	11.800	1.125	1.866	4.855	8.363
Turicibacteraceae	0.089	0.057	0.014	0.024	0.000	0.000	0.104	0.171
Veillonellaceae	1.615	1.238	2.195	1.336	1.254	0.617	2.034	0.680
Verrucomicrobiaceae	0.000	0.000	0.442	0.766	0.032	0.046	0.028	0.027
Victivallaceae	0.004	0.006	0.004	0.006	0.000	0.000	0.000	0.000

Genus	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
[Eubacterium]	1.442	2.458	1.087	1.757	1.532	2.383	3.237	5.311
[Prevotella]	0.009	0.016	0.036	0.063	0.254	0.440	0.231	0.384
[Ruminococcus]	0.560	0.463	0.751	0.431	0.948	0.089	0.658	0.548
Acidaminococcus	0.066	0.089	0.318	0.550	0.046	0.080	0.004	0.006
Acinetobacter	0.000	0.000	0.000	0.000	0.005	0.008	0.000	0.000
Actinomyces	0.005	0.009	0.014	0.013	0.015	0.015	0.011	0.019
Adlercreutzia	0.005	0.009	0.032	0.034	0.086	0.089	0.044	0.077
Akkermansia	0.000	0.000	0.612	1.059	0.044	0.063	0.039	0.042
Anaerofustis	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.000
Anaerostipes	0.158	0.152	0.131	0.065	0.113	0.089	0.071	0.070
Anaerotruncus	0.015	0.015	0.022	0.028	0.018	0.032	0.006	0.010
Atopobium	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.000
Bacteroides	29.714	20.404	36.800	9.190	34.484	7.478	39.557	11.796
Bifidobacterium	11.411	14.746	4.795	2.673	5.132	0.150	4.019	4.249
Bilophila	0.132	0.130	1.959	3.256	5.587	4.840	5.966	3.598
Blautia	4.589	2.529	6.416	4.302	5.530	2.504	3.873	2.023
Butyricimonas	0.282	0.400	0.369	0.429	0.230	0.299	0.164	0.195
cc_115	0.014	0.025	0.038	0.054	0.087	0.151	0.000	0.000
Christensenella	0.000	0.000	0.009	0.008	0.005	0.008	0.000	0.000
Citrobacter	0.030	0.041	0.000	0.000	0.616	1.053	0.009	0.008
Clostridium	2.541	3.350	0.078	0.081	0.052	0.041	0.112	0.112
Clostridium	0.000	0.000	0.004	0.008	0.000	0.000	0.006	0.010
Collinsella	0.666	0.889	0.856	0.911	1.309	1.295	0.639	0.492
Coprobacillus	0.026	0.044	0.009	0.016	0.000	0.000	0.066	0.115
Coprococcus	5.756	6.039	3.918	1.912	4.223	2.830	1.845	1.582
Corynebacterium	0.000	0.000	0.000	0.000	0.005	0.008	0.000	0.000
Curvibacter	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.000
Desulfovibrio	0.529	0.477	0.000	0.000	0.073	0.126	0.233	0.404
Dialister	1.155	1.895	2.326	1.415	0.987	0.866	1.372	2.376
Dorea	0.596	0.494	0.536	0.213	0.440	0.351	0.523	0.141
Eggerthella	0.041	0.071	0.004	0.008	0.000	0.000	0.006	0.010
Enterococcus	0.010	0.018	0.080	0.139	1.575	2.728	0.007	0.013
Epulopiscium	0.000	0.000	0.005	0.008	0.000	0.000	0.006	0.010
Escherichia	0.041	0.071	0.099	0.149	0.015	0.027	0.319	0.552
Faecalibacterium	4.183	3.056	3.632	2.027	6.924	1.241	5.181	3.764

Fusobacterium	0.000	0.000	0.000	0.000	0.000	0.000	9.519	16.487
Granulicatella	0.005	0.009	0.000	0.000	0.010	0.018	0.007	0.013
Haemophilus	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Helicobacter	0.000	0.000	0.000	0.000	0.010	0.018	0.000	0.000
Holdemania	0.035	0.017	0.070	0.071	0.080	0.070	0.094	0.047
Lachnobacterium	0.000	0.000	0.104	0.102	0.102	0.165	0.018	0.031
Lachnospira	1.777	2.614	0.607	0.812	1.039	0.784	0.600	0.714
Lactobacillus	3.001	5.158	1.563	2.464	0.194	0.325	0.395	0.670
Lactococcus	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Leuconostoc	0.000	0.000	0.013	0.023	0.000	0.000	0.007	0.013
Megasphaera	0.000	0.000	0.000	0.000	0.010	0.018	0.000	0.000
Mesorhizobium	0.005	0.008	0.000	0.000	0.009	0.008	0.006	0.010
Methanobrevibacter	0.066	0.115	0.168	0.187	0.032	0.056	0.000	0.000
Morganella	0.000	0.000	0.000	0.000	0.021	0.036	0.000	0.000
Odoribacter	0.276	0.372	0.423	0.276	0.349	0.121	0.411	0.278
Oscillospira	2.107	2.325	4.636	3.809	8.152	6.949	3.962	3.183
Other	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
p-75-a5	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.010
Parabacteroides	2.268	2.343	3.538	1.872	2.826	2.321	3.035	1.410
Paraprevotella	0.327	0.567	1.091	1.102	0.286	0.496	0.015	0.026
Peptococcus	0.185	0.320	0.144	0.250	0.245	0.424	0.000	0.000
Phascolarctobacterium	0.948	0.956	0.272	0.339	0.640	0.545	1.598	0.994
Prevotella	1.755	3.026	1.620	2.771	2.177	3.757	0.549	0.855
Pyramidobacter	0.000	0.000	0.337	0.583	0.000	0.000	0.000	0.000
Ralstonia	0.000	0.000	0.005	0.008	0.009	0.016	0.006	0.010
Roseburia	1.559	1.363	1.413	2.196	0.464	0.593	0.536	0.919
Rothia	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.013
Ruminococcus	6.383	5.277	9.175	1.666	4.993	3.658	1.835	1.683
Serratia	0.000	0.000	0.000	0.000	2.754	4.441	0.055	0.096
Slackia	0.019	0.033	0.113	0.075	0.072	0.025	0.048	0.074
SMB53	0.147	0.204	0.000	0.000	0.000	0.000	0.042	0.073
Staphylococcus	0.000	0.000	0.005	0.008	0.000	0.000	0.000	0.000
Streptococcus	12.852	20.921	8.700	14.974	1.684	2.806	5.393	9.266
Sutterella	2.147	1.860	1.040	0.580	3.440	2.534	3.471	2.991
Trabulsiella	0.000	0.000	0.000	0.000	0.005	0.008	0.000	0.000
Turicibacter	0.126	0.079	0.019	0.033	0.000	0.000	0.172	0.289
Veillonella	0.020	0.018	0.004	0.008	0.036	0.062	0.000	0.000

Table A15. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon VSL#3 treatment over time.

Phylum	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
Actinobacteria	17.827	17.686	11.415	13.823	3.370	1.263	2.983	1.014
Bacteroidetes	23.357	16.702	35.603	4.084	27.133	15.619	32.408	5.739
Cyanobacteria	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Euryarchaeota	0.000	0.000	0.037	0.064	0.017	0.029	0.074	0.119
Firmicutes	57.651	1.517	51.218	12.256	53.219	6.930	54.093	7.871
Fusobacteria	0.005	0.007	0.000	0.000	0.003	0.006	0.000	0.000
Lentisphaerae	0.000	0.000	0.000	0.000	0.013	0.015	0.010	0.017
Proteobacteria	1.139	0.554	1.700	1.773	16.235	14.171	10.424	8.040
Tenericutes	0.000	0.000	0.007	0.012	0.010	0.017	0.000	0.000
TM7	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Verrucomicrobia	0.020	0.029	0.020	0.027	0.000	0.000	0.000	0.000

Order	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
Actinomycetales	0.005	0.007	0.000	0.000	0.010	0.010	0.003	0.006
Bacteroidales	23.357	16.702	35.603	4.084	27.133	15.619	32.411	5.738
Bifidobacteriales	15.374	15.545	1.996	0.325	2.093	0.995	2.036	1.104
Burkholderiales	0.630	0.177	0.362	0.318	1.286	1.609	1.559	1.869
Clostridiales	42.416	0.501	39.858	6.650	40.887	8.826	41.709	4.663
Coriobacteriales	2.448	2.148	9.419	14.090	1.267	0.732	0.944	0.484
Desulfovibrionales	0.428	0.263	0.214	0.199	1.532	1.364	1.069	0.954
Enterobacteriales	0.076	0.107	1.116	1.340	13.404	11.817	7.791	8.112
Erysipelotrichales	1.340	1.223	2.811	0.773	6.642	6.442	1.599	1.041
Fusobacteriales	0.005	0.007	0.000	0.000	0.003	0.006	0.000	0.000
Lactobacillales	13.896	2.239	8.503	5.363	5.614	5.447	10.663	6.907
Methanobacteriales	0.000	0.000	0.037	0.064	0.017	0.029	0.074	0.119
Pasteurellales	0.005	0.007	0.003	0.006	0.010	0.017	0.003	0.006
RF39	0.000	0.000	0.007	0.012	0.010	0.017	0.000	0.000
Rhizobiales	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Turicibacterales	0.000	0.000	0.047	0.065	0.077	0.061	0.128	0.150
Verrucomicrobiales	0.020	0.029	0.017	0.029	0.000	0.000	0.000	0.000
Victivallales	0.000	0.000	0.000	0.000	0.013	0.015	0.010	0.017

Family	Time point							
	T0 mean ± sd		T5 mean ± sd		T10 mean ± sd		T24 mean ± sd	
[Barnesiellaceae]	0.130	0.125	0.593	0.305	0.402	0.099	0.623	0.344
[Cerasicoccaceae]	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.000
[Mogibacteriaceae]	0.058	0.023	0.190	0.154	0.150	0.105	0.346	0.306
[Odoribacteraceae]	0.168	0.164	0.348	0.187	0.487	0.277	0.504	0.152
[Paraprevotellaceae]	0.000	0.000	0.359	0.478	0.381	0.660	0.045	0.078
[Tissierellaceae]	0.005	0.007	0.000	0.000	0.055	0.087	0.019	0.032
Actinomycetaceae	0.005	0.007	0.000	0.000	0.011	0.011	0.004	0.006
Alcaligenaceae	0.653	0.180	0.373	0.322	1.341	1.663	1.665	2.012
Bacteroidaceae	17.217	9.750	28.011	1.973	21.561	11.269	26.304	2.464
Bifidobacteriaceae	15.894	16.024	2.097	0.358	2.218	1.038	2.166	1.167
Carnobacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.019
Christensenellaceae	0.010	0.015	0.050	0.051	0.043	0.023	0.044	0.049
Clostridiaceae	0.336	0.432	0.620	0.466	0.600	0.192	0.970	0.500
Coriobacteriaceae	2.532	2.212	9.645	14.360	1.361	0.798	1.004	0.493
Dehalobacteriaceae	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000
Desulfovibrionaceae	0.443	0.270	0.225	0.205	1.640	1.477	1.165	1.045
Enterobacteriaceae	0.078	0.110	1.145	1.365	14.248	12.463	8.493	9.016
Enterococcaceae	4.803	6.762	0.250	0.432	0.011	0.010	0.007	0.013
Erysipelotrichaceae	1.385	1.260	2.965	0.910	6.969	6.607	1.711	1.122
Eubacteriaceae	0.000	0.000	0.027	0.047	0.000	0.000	0.000	0.000
Fusobacteriaceae	0.005	0.007	0.000	0.000	0.003	0.006	0.000	0.000
Lachnospiraceae	21.848	1.929	17.831	2.749	15.312	8.411	14.342	2.104
Lactobacillaceae	1.852	1.678	1.201	0.743	0.893	0.865	1.117	0.673
Leuconostocaceae	0.000	0.000	0.007	0.012	0.000	0.000	0.000	0.000
Methanobacteriaceae	0.000	0.000	0.038	0.067	0.018	0.031	0.077	0.123
Oxalobacteraceae	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000
Pasteurellaceae	0.005	0.007	0.003	0.006	0.011	0.019	0.004	0.006
Peptococcaceae	0.000	0.000	0.581	1.005	0.178	0.299	0.699	1.182
Peptostreptococcaceae	0.000	0.000	0.004	0.006	0.114	0.171	0.244	0.377
Phyllobacteriaceae	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.000
Porphyromonadaceae	0.892	0.939	3.088	3.026	2.710	2.156	3.366	2.819
Prevotellaceae	0.005	0.007	3.034	2.642	1.402	2.314	1.463	1.283
Rikenellaceae	0.788	0.430	1.524	1.008	1.776	0.880	1.960	0.149
Ruminococcaceae	16.939	1.860	15.455	6.477	19.432	4.093	18.577	6.105
S24-7	5.074	7.175	0.390	0.676	0.343	0.594	0.304	0.516

Streptococcaceae	7.731	7.522	7.579	5.655	5.025	4.776	10.285	6.778
Turicibacteraceae	0.000	0.000	0.051	0.070	0.082	0.063	0.138	0.160
Veillonellaceae	1.123	0.235	2.292	0.229	1.201	0.384	2.336	1.075
Verrucomicrobiaceae	0.021	0.030	0.017	0.030	0.000	0.000	0.000	0.000
Victivallaceae	0.000	0.000	0.000	0.000	0.014	0.016	0.011	0.019

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	0.014	0.019	3.228	1.028	1.956	2.072	1.229	1.895
[Prevotella]	0.000	0.000	0.323	0.456	0.294	0.416	0.214	0.370
[Ruminococcus]	1.079	0.159	1.017	1.191	0.856	0.244	1.604	1.600
Acidaminococcus	0.366	0.517	0.000	0.000	0.105	0.149	0.000	0.000
Actinomyces	0.008	0.011	0.000	0.000	0.025	0.003	0.015	0.026
Adlercreutzia	0.070	0.055	0.000	0.000	0.023	0.033	0.284	0.418
Akkermansia	0.031	0.043	0.000	0.000	0.000	0.000	0.005	0.009
Anaerofustis	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.018
Anaerostipes	0.076	0.088	0.048	0.027	0.107	0.119	0.085	0.061
Anaerotruncus	0.000	0.000	0.000	0.000	0.019	0.007	0.041	0.071
Atopobium	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.008
Bacteroides	24.321	15.372	35.504	2.740	29.779	13.677	31.992	9.016
Bifidobacterium	21.069	20.377	3.007	0.141	4.570	0.580	14.084	19.444
Bilophila	0.521	0.432	0.184	0.261	1.824	2.579	1.453	1.577
Blautia	7.382	1.476	7.145	1.813	7.249	1.920	8.079	1.918
Butyricimonas	0.007	0.010	0.376	0.115	0.313	0.409	0.236	0.232
cc_115	0.000	0.000	0.227	0.284	0.007	0.010	0.009	0.016
Christensenella	0.007	0.010	0.007	0.010	0.000	0.000	0.005	0.009
Citrobacter	0.007	0.010	0.000	0.000	0.035	0.050	0.000	0.000
Clostridium	0.015	0.022	0.333	0.366	0.146	0.091	0.075	0.077
Clostridium	0.000	0.000	0.000	0.000	0.012	0.017	0.000	0.000
Collinsella	3.018	3.031	1.081	1.362	1.027	1.122	3.617	4.524
Coprobacillus	0.000	0.000	0.000	0.000	0.035	0.050	0.005	0.009
Coprococcus	7.836	3.095	4.157	0.174	5.556	3.559	7.967	1.612
Dehalobacterium	0.000	0.000	0.000	0.000	0.007	0.010	0.009	0.016
Desulfovibrio	0.061	0.087	0.000	0.000	0.409	0.579	0.025	0.043
Dialister	0.224	0.316	2.348	0.065	0.780	1.103	0.614	0.747
Dorea	0.951	0.455	1.093	0.585	0.848	0.504	1.707	0.745
Enterococcus	6.192	8.735	0.000	0.000	0.012	0.017	1.237	2.142

Escherichia	0.007	0.010	0.007	0.010	0.327	0.463	0.045	0.055
Faecalibacterium	4.656	4.593	3.847	0.703	5.271	2.164	3.302	3.076
Fusobacterium	0.007	0.010	0.000	0.000	0.012	0.017	0.000	0.000
Holdemania	0.000	0.000	0.007	0.010	0.035	0.050	0.036	0.062
Lachnobacterium	0.102	0.144	0.044	0.043	0.007	0.010	0.005	0.008
Lachnospira	1.548	1.826	0.207	0.292	1.156	1.539	0.392	0.211
Lactobacillus	2.516	2.389	1.750	1.011	1.760	2.470	0.222	0.359
Lactococcus	0.000	0.000	0.000	0.000	0.012	0.017	0.000	0.000
Leuconostoc	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.079
Megamonas	0.007	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Megasphaera	0.014	0.019	0.007	0.010	0.000	0.000	0.000	0.000
Mesorhizobium	0.000	0.000	0.007	0.010	0.000	0.000	0.000	0.000
Methanobrevibacter	0.000	0.000	0.074	0.105	0.034	0.048	0.009	0.016
Odoribacter	0.234	0.255	0.224	0.164	0.292	0.084	0.220	0.139
Oscillospira	0.631	0.046	2.280	1.701	3.975	3.339	4.373	5.519
Parabacteroides	1.285	1.395	5.687	3.597	2.912	3.390	3.624	2.367
Paraprevotella	0.000	0.000	0.256	0.361	0.438	0.619	0.341	0.591
Parvimonas	0.000	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Pediococcus	0.008	0.011	0.000	0.000	0.000	0.000	0.000	0.000
Peptococcus	0.000	0.000	1.116	1.579	0.335	0.474	0.136	0.236
Peptoniphilus	0.007	0.010	0.000	0.000	0.000	0.000	0.005	0.009
Phascolarctobacterium	0.698	0.988	0.703	0.222	1.076	0.825	0.873	0.391
Prevotella	0.007	0.010	5.978	0.294	2.607	3.686	1.551	2.686
Pseudomonas	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.009
Ralstonia	0.000	0.000	0.000	0.000	0.007	0.010	0.000	0.000
Roseburia	1.186	1.600	0.115	0.067	0.210	0.032	0.171	0.101
Ruminococcus	1.582	0.522	3.111	1.248	8.643	1.929	5.593	4.659
Serratia	0.007	0.010	0.000	0.000	0.094	0.132	0.000	0.000
Slackia	0.037	0.034	0.027	0.038	0.048	0.068	0.242	0.373
SMB53	0.000	0.000	0.066	0.094	0.035	0.050	0.005	0.008
Streptococcus	11.094	11.245	14.102	3.892	10.708	15.046	1.905	2.316
Sutterella	0.871	0.147	0.206	0.166	3.827	4.871	2.237	3.353
Turicibacter	0.000	0.000	0.102	0.106	0.177	0.212	0.000	0.000
Veillonella	0.230	0.326	0.000	0.000	0.000	0.000	0.000	0.000

Table A16. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon VSL#3 + lactulose treatment, over time.

Phylum	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinobacteria	3.866	2.553	18.398	9.928	13.158	15.403	23.039	19.096
Bacteroidetes	35.434	1.796	26.316	6.300	30.532	10.158	22.240	10.424
Chloroflexi	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyanobacteria	0.003	0.006	0.003	0.006	0.003	0.006	0.000	0.000
Euryarchaeota	0.000	0.000	0.010	0.017	0.007	0.012	0.077	0.134
Firmicutes	52.158	5.312	53.081	10.787	53.076	5.978	53.809	9.923
Fusobacteria	0.101	0.175	0.000	0.000	0.000	0.000	0.000	0.000
Lentisphaerae	0.000	0.000	0.000	0.000	0.007	0.006	0.000	0.000
Proteobacteria	8.414	9.318	2.192	1.510	3.211	4.189	0.821	0.464
Tenericutes	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
TM7	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Verrucomicrobia	0.020	0.017	0.000	0.000	0.003	0.006	0.013	0.023

Order	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinomycetales	0.010	0.010	0.007	0.012	0.010	0.017	0.000	0.000
Bacteroidales	35.436	1.792	26.317	6.300	30.533	10.160	22.240	10.424
Bifidobacteriales	2.953	2.459	16.506	7.525	9.655	13.207	20.633	17.665
Burkholderiales	1.123	1.394	1.320	1.093	1.506	2.225	0.484	0.674
Clostridiales	35.821	3.087	45.137	12.374	47.443	9.566	31.223	12.562
Coriobacteriales	0.903	0.090	1.886	2.572	3.492	2.254	2.406	3.670
Desulfovibrionales	0.756	0.804	0.584	0.497	0.983	1.035	0.239	0.266
Enterobacteriales	6.520	7.246	0.288	0.207	0.718	0.985	0.097	0.061
Erysipelotrichales	6.302	9.807	5.143	3.766	3.282	3.084	18.353	21.061
Fusobacteriales	0.101	0.175	0.000	0.000	0.000	0.000	0.000	0.000
Gemellales	0.007	0.012	0.000	0.000	0.000	0.000	0.000	0.000
Lactobacillales	10.028	6.516	2.622	0.990	2.350	2.010	4.233	1.974
Methanobacteriales	0.000	0.000	0.010	0.017	0.007	0.012	0.077	0.134
Pseudomonadales	0.003	0.006	0.000	0.000	0.003	0.006	0.000	0.000
RF32	0.007	0.006	0.000	0.000	0.000	0.000	0.000	0.000
RF39	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacterales	0.003	0.006	0.181	0.313	0.000	0.000	0.000	0.000

Verrucomicrobiales	0.020	0.017	0.000	0.000	0.003	0.006	0.013	0.023
Victivallales	0.000	0.000	0.000	0.000	0.007	0.006	0.000	0.000
YS2	0.003	0.006	0.000	0.000	0.003	0.006	0.000	0.000

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.115	0.139	0.086	0.106	0.237	0.375	0.248	0.413
[Mogibacteriaceae]	0.017	0.016	0.052	0.018	0.553	0.850	0.071	0.071
[Odoribacteraceae]	0.319	0.166	0.114	0.104	0.335	0.240	0.089	0.067
[Paraprevotellaceae]	0.745	1.290	0.187	0.324	0.432	0.749	0.058	0.100
[Tissierellaceae]	0.007	0.012	0.000	0.000	0.003	0.006	0.000	0.000
Actinomycetaceae	0.007	0.006	0.004	0.006	0.010	0.018	0.000	0.000
Alcaligenaceae	1.137	1.397	1.385	1.178	1.573	2.319	0.493	0.680
Bacteroidaceae	25.669	5.534	20.762	2.098	23.452	7.543	18.528	12.865
Bifidobacteriaceae	3.067	2.567	17.337	8.075	10.202	13.972	21.494	18.209
Carnobacteriaceae	0.007	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Christensenellaceae	0.014	0.016	0.017	0.029	0.259	0.430	0.037	0.056
Clostridiaceae	0.522	0.314	1.999	3.042	0.715	0.526	0.130	0.096
Coriobacteriaceae	0.931	0.109	1.983	2.713	3.679	2.392	2.590	3.984
Dehalobacteriaceae	0.000	0.000	0.003	0.006	0.007	0.012	0.000	0.000
Desulfovibrionaceae	0.768	0.804	0.619	0.527	1.030	1.079	0.245	0.266
Enterobacteriaceae	6.623	7.275	0.305	0.222	0.750	1.027	0.102	0.067
Enterococcaceae	0.024	0.026	0.036	0.053	0.875	1.496	0.040	0.060
Erysipelotrichaceae	6.561	10.227	5.318	3.809	3.466	3.263	18.645	21.000
Fusobacteriaceae	0.102	0.176	0.000	0.000	0.000	0.000	0.000	0.000
Gemellaceae	0.007	0.012	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	17.336	4.166	19.366	1.324	22.532	0.900	16.919	8.628
Lactobacillaceae	1.545	0.749	0.572	0.043	0.174	0.283	0.557	0.337
Leuconostocaceae	0.000	0.000	0.000	0.000	0.031	0.055	0.000	0.000
Methanobacteriaceae	0.000	0.000	0.010	0.018	0.007	0.012	0.078	0.136
Microbacteriaceae	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Oxalobacteraceae	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.000
Pasteurellaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Peptococcaceae	0.000	0.000	0.109	0.189	0.106	0.184	0.126	0.218
Peptostreptococcaceae	0.028	0.048	0.011	0.019	0.014	0.016	0.003	0.006
Porphyromonadaceae	2.144	0.531	1.924	2.281	2.666	1.805	1.587	2.229
Prevotellaceae	3.014	3.134	0.753	1.303	1.208	2.093	0.565	0.960
Pseudomonadaceae	0.003	0.006	0.000	0.000	0.003	0.006	0.000	0.000

Rikenellaceae	0.762	0.219	0.928	0.226	2.081	1.798	0.788	0.735
Ruminococcaceae	13.597	2.429	19.738	10.352	19.418	8.552	10.755	2.311
S24-7	3.732	5.530	2.673	4.629	1.705	2.954	0.889	1.532
Streptococcaceae	8.743	6.085	2.118	1.054	1.360	1.674	3.802	1.764
Turicibacteraceae	0.003	0.006	0.193	0.334	0.000	0.000	0.000	0.000
Veillonellaceae	2.428	2.117	1.391	0.903	1.094	0.382	1.144	0.960
Verrucomicrobiaceae	0.021	0.018	0.000	0.000	0.004	0.006	0.015	0.025
Victivallaceae	0.000	0.000	0.000	0.000	0.007	0.006	0.000	0.000

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	3.207	5.438	2.749	4.749	1.229	1.895	3.418	3.124
[Prevotella]	0.997	1.726	0.116	0.200	0.214	0.370	0.060	0.104
[Ruminococcus]	0.680	0.500	0.596	0.283	1.604	1.600	1.685	2.061
Acidaminococcus	0.636	1.102	0.351	0.608	0.000	0.000	0.000	0.000
Actinomyces	0.005	0.009	0.005	0.009	0.015	0.026	0.000	0.000
Adlercreutzia	0.030	0.039	0.015	0.026	0.284	0.418	0.042	0.049
Akkermansia	0.030	0.026	0.000	0.000	0.005	0.009	0.020	0.035
Anaerofustis	0.000	0.000	0.000	0.000	0.010	0.018	0.000	0.000
Anaerostipes	0.101	0.120	0.128	0.100	0.085	0.061	0.031	0.053
Anaerotruncus	0.005	0.009	0.000	0.000	0.041	0.071	0.013	0.022
Atopobium	0.000	0.000	0.000	0.000	0.005	0.008	0.000	0.000
Bacteroides	38.511	9.860	27.521	0.558	31.992	9.016	31.065	28.115
Bifidobacterium	4.528	3.642	23.569	12.160	14.084	19.444	28.647	23.559
Bilophila	1.072	1.243	0.420	0.642	1.453	1.577	0.417	0.524
Blautia	4.815	1.517	6.137	1.994	8.079	1.918	5.294	1.925
Butyricimonas	0.163	0.203	0.054	0.093	0.236	0.232	0.032	0.040
cc_115	0.011	0.019	0.005	0.009	0.009	0.016	0.000	0.000
Christensenella	0.000	0.000	0.004	0.007	0.005	0.009	0.004	0.007
Citrobacter	0.005	0.010	0.010	0.017	0.000	0.000	0.000	0.000
Clostridium	0.281	0.446	0.307	0.378	0.075	0.077	0.009	0.008
Clostridium	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.022
Collinsella	0.974	0.143	2.467	3.872	3.617	4.524	3.436	5.561
Coprobacillus	0.005	0.009	0.044	0.077	0.005	0.009	0.097	0.167
Coprococcus	3.625	0.495	4.466	1.257	7.967	1.612	3.979	2.058
Cryocola	0.005	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Dehalobacterium	0.000	0.000	0.004	0.007	0.009	0.016	0.000	0.000

Desulfovibrio	0.060	0.103	0.440	0.674	0.025	0.043	0.015	0.027
Dialister	0.638	1.105	0.428	0.434	0.614	0.747	0.309	0.270
Dorea	0.807	0.521	1.624	1.299	1.707	0.745	1.297	1.222
Eggerthella	0.075	0.130	0.168	0.291	0.056	0.097	0.000	0.000
Enterococcus	0.025	0.043	0.000	0.000	1.237	2.142	0.012	0.010
Epulopiscium	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Escherichia	0.324	0.339	0.010	0.017	0.045	0.055	0.000	0.000
Faecalibacterium	5.182	0.966	6.782	4.556	3.302	3.076	2.469	1.687
Finegoldia	0.011	0.019	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacterium	0.150	0.260	0.000	0.000	0.000	0.000	0.000	0.000
Granulicatella	0.010	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Holdemania	0.016	0.015	0.030	0.026	0.036	0.062	0.090	0.156
Klebsiella	0.095	0.165	0.000	0.000	0.000	0.000	0.000	0.000
Lachnobacterium	0.011	0.019	0.000	0.000	0.005	0.008	0.019	0.033
Lachnospira	1.064	1.566	0.949	1.189	0.392	0.211	0.319	0.357
Lactobacillus	2.060	0.997	0.696	0.153	0.222	0.359	0.763	0.594
Leuconostoc	0.000	0.000	0.000	0.000	0.046	0.079	0.000	0.000
Methanobrevibacter	0.000	0.000	0.012	0.021	0.009	0.016	0.099	0.171
Odoribacter	0.310	0.130	0.093	0.097	0.220	0.139	0.097	0.044
Oscillospira	1.870	1.768	1.117	0.769	4.373	5.519	2.267	2.341
Parabacteroides	3.208	0.829	2.417	2.740	3.624	2.367	2.039	2.775
Paraprevotella	0.105	0.182	0.112	0.193	0.341	0.591	0.013	0.022
Peptococcus	0.000	0.000	0.132	0.229	0.136	0.236	0.159	0.275
Peptoniphilus	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.000
Peptostreptococcus	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Phascolarctobacterium	2.332	2.537	1.025	0.528	0.873	0.391	1.291	1.578
Prevotella	4.547	4.631	0.913	1.582	1.551	2.686	0.713	1.209
Pseudomonas	0.005	0.009	0.000	0.000	0.005	0.009	0.000	0.000
Roseburia	0.784	1.261	2.510	4.094	0.171	0.101	0.335	0.457
Ruminococcus	2.140	1.352	6.553	5.429	5.593	4.659	2.844	2.168
Scardovia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Slackia	0.015	0.026	0.032	0.030	0.242	0.373	0.056	0.049
SMB53	0.005	0.010	0.088	0.142	0.005	0.008	0.000	0.000
Streptococcus	12.757	8.472	2.762	1.202	1.905	2.316	5.970	3.193
Sutterella	1.680	2.068	1.801	1.700	2.237	3.353	0.562	0.707
Turicibacter	0.005	0.010	0.267	0.462	0.000	0.000	0.000	0.000
Varibaculum	0.005	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Veillonella	0.010	0.009	0.054	0.094	0.000	0.000	0.000	0.000

Table A17. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon VSL#3 + lactulose + rifaximin treatment. over time.

Phylum	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinobacteria	2.719	0.991	3.345	1.506	2.604	2.108	3.188	3.461
Bacteroidetes	25.768	10.080	23.664	4.912	28.718	24.674	21.806	3.715
Chloroflexi	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyanobacteria	0.000	0.000	0.003	0.006	0.007	0.012	0.003	0.006
Euryarchaeota	0.013	0.023	0.044	0.076	0.020	0.035	0.132	0.228
Firmicutes	70.680	11.251	53.765	21.836	61.251	30.984	50.611	25.571
Fusobacteria	0.000	0.000	0.000	0.000	0.194	0.337	0.000	0.000
Lentisphaerae	0.010	0.010	0.000	0.000	0.003	0.006	0.000	0.000
Proteobacteria	0.779	0.450	19.159	21.691	7.129	9.822	24.252	20.676
Tenericutes	0.030	0.052	0.000	0.000	0.000	0.000	0.000	0.000
Verrucomicrobia	0.000	0.000	0.020	0.020	0.074	0.128	0.007	0.006

Order	Time point							
	T0		T5		T10		T24	
	mean ± sd		mean ± sd		mean ± sd		mean ± sd	
Actinomycetales	1.904	0.655	0.007	0.012	0.010	0.017	0.000	0.000
Bacteroidales	0.000	0.000	23.664	4.912	28.718	24.674	21.808	3.713
Bifidobacteriales	0.788	0.472	2.739	1.609	1.443	1.150	2.919	3.535
Burkholderiales	0.000	0.000	0.511	0.646	0.958	1.249	2.447	2.441
Campylobacterales	0.000	0.000	0.007	0.012	0.000	0.000	0.000	0.000
Cardiobacteriales	0.138	0.111	0.000	0.000	0.000	0.000	0.000	0.000
Clostridiales	0.003	0.006	27.867	15.120	28.988	17.750	26.637	12.833
Coriobacteriales	25.768	10.080	0.599	0.306	1.151	0.983	0.270	0.092
Desulfovibrionales	0.000	0.000	1.697	1.862	1.170	1.020	0.437	0.394
Enterobacteriales	0.000	0.000	16.928	19.235	4.924	7.995	21.354	18.663
Erysipelotrichales	0.000	0.000	21.757	18.404	28.557	46.104	18.249	15.883
Fusobacteriales	0.010	0.010	0.000	0.000	0.194	0.337	0.000	0.000
Gemellales	10.210	12.016	0.000	0.000	0.000	0.000	0.000	0.000
I025	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Lactobacillales	0.000	0.000	4.077	1.565	3.699	3.823	5.724	3.899
Methanobacteriales	0.027	0.046	0.044	0.076	0.020	0.035	0.132	0.228
Neisseriales	0.228	0.319	0.000	0.000	0.023	0.041	0.000	0.000
Pasteurellales	0.000	0.000	0.000	0.000	0.047	0.081	0.003	0.006

RF32	0.000	0.000	0.010	0.017	0.003	0.006	0.003	0.006
RF39	0.027	0.047	0.000	0.000	0.000	0.000	0.000	0.000
Rhizobiales	0.000	0.000	0.007	0.006	0.003	0.006	0.000	0.000
SHA-98	15.576	25.180	0.000	0.000	0.000	0.000	0.000	0.000
Sphingomonadales	0.397	0.305	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacterales	44.890	10.460	0.064	0.111	0.013	0.015	0.007	0.012
Verrucomicrobiales	0.000	0.000	0.020	0.020	0.074	0.128	0.007	0.006
Victivallales	0.017	0.021	0.000	0.000	0.003	0.006	0.000	0.000
YS2	0.000	0.000	0.003	0.006	0.000	0.000	0.003	0.006

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.052	0.021	0.191	0.167	0.314	0.379	0.157	0.222
[Mogibacteriaceae]	0.350	0.228	0.059	0.093	0.098	0.152	0.061	0.081
[Odoribacteraceae]	0.583	0.481	0.132	0.110	0.311	0.383	0.127	0.078
[Paraprevotellaceae]	0.293	0.508	0.236	0.400	0.415	0.719	0.276	0.478
Actinomycetaceae	0.025	0.043	0.007	0.012	0.007	0.012	0.000	0.000
Alcaligenaceae	0.413	0.322	0.527	0.668	0.977	1.263	2.512	2.534
Bacteroidaceae	21.011	8.417	21.569	6.902	24.638	21.457	20.255	4.684
Bifidobacteriaceae	1.981	0.709	2.813	1.679	1.499	1.212	3.002	3.661
Carnobacteriaceae	0.000	0.000	0.003	0.006	0.000	0.000	0.000	0.000
Christensenellaceae	0.351	0.295	0.003	0.006	0.011	0.018	0.003	0.006
Clostridiaceae	0.199	0.236	0.683	0.668	0.539	0.471	1.539	2.242
Coriobacteriaceae	0.822	0.500	0.612	0.315	1.192	1.029	0.274	0.091
Corynebacteriaceae	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000
Dehalobacteriaceae	0.000	0.000	0.003	0.006	0.007	0.012	0.000	0.000
Desulfovibrionaceae	0.239	0.334	1.739	1.927	1.203	1.052	0.448	0.406
Enterobacteriaceae	0.144	0.116	17.362	19.908	4.998	8.100	21.869	19.175
Enterococcaceae	0.109	0.123	0.042	0.072	0.335	0.391	0.938	1.476
Erysipelotrichaceae	15.800	25.481	22.071	18.538	29.747	48.028	18.459	16.038
Eubacteriaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.012
Fusobacteriaceae	0.000	0.000	0.000	0.000	0.197	0.341	0.000	0.000
Gemellaceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Helicobacteraceae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lachnospiraceae	20.032	1.973	10.386	8.472	11.867	7.436	13.474	15.012
Lactobacillaceae	1.140	1.551	1.019	0.177	0.490	0.742	1.322	1.570
Leuconostocaceae	0.046	0.040	0.038	0.066	0.020	0.035	0.051	0.089

Methanobacteriaceae	0.014	0.024	0.045	0.078	0.021	0.037	0.133	0.230
Neisseriaceae	0.000	0.000	0.000	0.000	0.024	0.041	0.000	0.000
Pasteurellaceae	0.000	0.000	0.000	0.000	0.048	0.082	0.003	0.006
Peptococcaceae	0.027	0.047	0.073	0.126	0.120	0.207	0.041	0.071
Peptostreptococcaceae	0.000	0.000	0.003	0.006	0.024	0.021	0.038	0.066
Phyllobacteriaceae	0.000	0.000	0.007	0.006	0.004	0.006	0.000	0.000
Porphyromonadaceae	0.926	0.639	0.575	0.699	1.908	2.514	0.184	0.181
Prevotellaceae	0.583	1.001	0.885	1.523	1.273	2.205	0.702	1.208
Rikenellaceae	3.364	2.763	0.412	0.482	0.744	1.137	0.542	0.588
Ruminococcaceae	21.219	11.593	13.879	5.347	11.986	9.428	6.819	4.329
S24-7	0.000	0.000	0.128	0.222	0.003	0.006	0.000	0.000
Streptococcaceae	9.237	11.089	3.053	1.856	2.936	2.784	3.491	3.091
Turicibacteraceae	0.000	0.000	0.066	0.114	0.014	0.016	0.007	0.012
Veillonellaceae	1.028	0.202	1.350	0.526	1.947	1.896	3.256	4.311
Verrucomicrobiaceae	0.000	0.000	0.020	0.020	0.075	0.129	0.007	0.006
Victivallaceae	0.010	0.011	0.000	0.000	0.004	0.006	0.000	0.000

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	7.166	11.752	3.138	4.505	5.471	6.398	3.418	3.124
[Prevotella]	0.000	0.000	0.034	0.046	0.183	0.316	0.060	0.104
[Ruminococcus]	1.003	0.580	0.483	0.025	0.970	0.871	1.685	2.061
Acidaminococcus	0.005	0.008	0.065	0.112	0.000	0.000	0.000	0.000
Actinomyces	0.034	0.060	0.010	0.017	0.009	0.015	0.000	0.000
Adlercreutzia	0.145	0.165	0.006	0.011	0.000	0.000	0.042	0.049
Akkermansia	0.000	0.000	0.035	0.038	0.095	0.164	0.020	0.035
Anaerostipes	0.130	0.095	0.041	0.056	0.101	0.100	0.031	0.053
Anaerotruncus	0.020	0.034	0.006	0.010	0.004	0.008	0.013	0.022
Atopobium	0.000	0.000	0.000	0.000	0.031	0.054	0.000	0.000
Bacteroides	31.250	9.128	36.392	15.648	31.738	26.326	31.065	28.115
Bifidobacterium	2.915	0.507	4.418	2.139	3.623	2.416	28.647	23.559
Bilophila	0.331	0.470	2.646	2.665	6.535	9.553	0.417	0.524
Blautia	7.798	1.388	4.239	4.040	4.612	2.267	5.294	1.925
Butyricimonas	0.248	0.163	0.098	0.171	0.254	0.440	0.032	0.040
Campylobacter	0.000	0.000	0.010	0.017	0.000	0.000	0.000	0.000
cc_115	0.000	0.000	0.015	0.026	0.063	0.109	0.000	0.000
Christensenella	0.007	0.012	0.000	0.000	0.004	0.008	0.004	0.007

Citrobacter	0.010	0.017	12.981	14.427	2.020	3.498	0.000	0.000
Clostridium	0.104	0.155	0.186	0.157	0.035	0.030	0.009	0.008
Clostridium	0.000	0.000	0.000	0.000	0.031	0.054	0.013	0.022
Collinsella	0.155	0.182	0.801	0.567	1.231	0.827	3.436	5.561
Coprobacillus	0.098	0.170	0.015	0.026	0.887	1.513	0.097	0.167
Coprococcus	10.149	9.057	5.877	8.220	3.663	2.730	3.979	2.058
Corynebacterium	0.000	0.000	0.000	0.000	0.004	0.008	0.000	0.000
Dehalobacterium	0.000	0.000	0.006	0.010	0.009	0.015	0.000	0.000
Desulfovibrio	0.000	0.000	0.052	0.061	0.000	0.000	0.015	0.027
Dialister	1.411	0.598	0.986	1.644	0.866	0.875	0.309	0.270
Dorea	0.586	0.466	0.434	0.292	0.541	0.457	1.297	1.222
Eggerthella	0.009	0.008	0.035	0.060	0.082	0.142	0.000	0.000
Enterococcus	0.140	0.166	0.025	0.043	1.010	1.089	0.012	0.010
Erwinia	0.000	0.000	0.005	0.009	0.004	0.007	0.000	0.000
Escherichia	0.000	0.000	0.000	0.000	0.164	0.193	0.000	0.000
Faecalibacterium	1.598	0.165	3.282	3.129	2.811	3.372	2.469	1.687
Fusobacterium	0.000	0.000	0.000	0.000	0.250	0.433	0.000	0.000
Granulicatella	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Haemophilus	0.000	0.000	0.000	0.000	0.060	0.104	0.000	0.000
Holdemania	0.123	0.138	0.155	0.228	0.730	1.242	0.090	0.156
Klebsiella	0.000	0.000	0.000	0.000	0.013	0.022	0.000	0.000
Lachnobacterium	0.139	0.171	0.012	0.020	0.000	0.000	0.019	0.033
Lachnospira	0.673	0.583	0.599	0.534	0.131	0.129	0.319	0.357
Lactobacillus	1.459	1.813	1.544	0.172	0.735	0.821	0.763	0.594
Lactococcus	0.000	0.000	0.005	0.009	0.004	0.007	0.000	0.000
Leuconostoc	0.062	0.055	0.000	0.000	0.000	0.000	0.000	0.000
Megasphaera	0.000	0.000	0.000	0.000	0.009	0.015	0.000	0.000
Mesorhizobium	0.000	0.000	0.012	0.011	0.004	0.008	0.000	0.000
Methanobrevibacter	0.027	0.047	0.075	0.130	0.027	0.046	0.099	0.171
Morganella	0.000	0.000	0.447	0.774	0.267	0.462	0.000	0.000
Odoribacter	0.557	0.500	0.112	0.078	0.140	0.126	0.097	0.044
Oscillospira	4.451	4.578	2.912	0.598	5.296	5.370	2.267	2.341
Parabacteroides	1.293	0.808	0.943	1.177	2.417	3.184	2.039	2.775
Paraprevotella	0.579	1.003	0.359	0.622	0.343	0.594	0.013	0.022
Peptococcus	0.054	0.093	0.122	0.211	0.151	0.262	0.159	0.275
Phascolarctobacterium	0.210	0.081	1.134	0.760	1.584	2.126	1.291	1.578
Prevotella	1.150	1.979	1.476	2.544	1.612	2.792	0.713	1.209
Proteus	0.000	0.000	0.000	0.000	0.004	0.007	0.000	0.000

Roseburia	1.606	1.681	0.632	1.065	0.053	0.053	0.335	0.457
Rothia	0.005	0.009	0.000	0.000	0.000	0.000	0.000	0.000
Ruminococcus	8.898	5.914	2.941	2.152	5.248	2.867	2.844	2.168
Serratia	0.000	0.000	4.048	5.547	0.090	0.157	0.000	0.000
Slackia	0.194	0.182	0.012	0.011	0.027	0.046	0.056	0.049
SMB53	0.000	0.000	0.020	0.034	0.157	0.272	0.000	0.000
Streptococcus	12.744	13.779	5.234	3.315	11.690	14.427	5.970	3.193
Sutterella	0.448	0.494	0.717	0.980	1.481	1.404	0.562	0.707
Trabulsiella	0.000	0.000	0.010	0.017	0.000	0.000	0.000	0.000
Turicibacter	0.000	0.000	0.094	0.163	0.099	0.159	0.000	0.000
Veillonella	0.016	0.014	0.055	0.095	0.297	0.515	0.000	0.000

Table A18. Mean relative abundance (%) ± standard deviation (SD) of bacterial taxa at the phylum, order, family and genus levels in Healthy subjects (HS) upon VSL#3 + rifaximin treatment over time.

	Time point							
Phylum	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
Actinobacteria	3.548	1.395	2.571	2.268	4.093	1.558	3.060	0.677
Bacteroidetes	35.957	2.121	34.111	11.109	32.075	8.075	33.183	0.274
Cyanobacteria	0.010	0.017	0.017	0.021	0.000	0.000	0.010	0.010
Euryarchaeota	0.050	0.087	0.000	0.000	0.003	0.006	0.000	0.000
Firmicutes	58.993	2.428	48.425	15.816	51.750	8.207	52.445	5.015
Fusobacteria	0.000	0.000	0.054	0.093	0.000	0.000	0.007	0.012
Lentisphaerae	0.017	0.021	0.000	0.000	0.000	0.000	0.010	0.017
Proteobacteria	1.418	0.882	14.755	23.114	11.991	12.262	11.265	4.691
Synergistetes	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Tenericutes	0.003	0.006	0.003	0.006	0.007	0.012	0.007	0.006
TM7	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Verrucomicrobia	0.000	0.000	0.064	0.086	0.081	0.073	0.010	0.000

	Time point							
Order	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Cerasicoccales]	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006
Actinomycetales	0.007	0.006	0.003	0.006	0.007	0.012	0.003	0.006
Bacteroidales	35.957	2.121	34.111	11.109	32.076	8.074	33.187	0.275
Bifidobacteriales	2.693	1.112	2.174	2.153	3.197	1.391	2.157	0.175
Burkholderiales	0.466	0.465	0.885	0.759	0.584	0.455	2.709	3.088

Campylobacterales	0.000	0.000	0.010	0.017	0.017	0.029	0.003	0.006
Clostridiales	46.626	11.299	30.118	12.732	39.591	12.685	41.018	3.872
Coriobacteriales	0.848	0.320	0.393	0.304	0.890	0.327	0.900	0.553
Desulfovibrionales	0.835	0.761	1.570	1.838	2.609	2.394	2.445	1.645
Enterobacteriales	0.114	0.082	12.284	20.997	8.762	10.323	6.095	5.190
Erysipelotrichales	2.764	2.136	12.499	16.971	2.881	3.760	2.118	3.259
Fusobacteriales	0.000	0.000	0.054	0.093	0.000	0.000	0.007	0.012
Lactobacillales	9.479	6.976	5.717	6.726	9.246	2.759	9.254	4.437
Methanobacteriales	0.050	0.087	0.000	0.000	0.003	0.006	0.000	0.000
Pasteurellales	0.003	0.006	0.000	0.000	0.007	0.012	0.013	0.006
Pseudomonadales	0.000	0.000	0.000	0.000	0.007	0.012	0.000	0.000
RF32	0.000	0.000	0.007	0.006	0.000	0.000	0.000	0.000
RF39	0.003	0.006	0.003	0.006	0.007	0.012	0.007	0.006
Rhizobiales	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Synergistales	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Turicibacterales	0.124	0.156	0.091	0.086	0.034	0.058	0.060	0.056
Verrucomicrobiales	0.000	0.000	0.064	0.086	0.081	0.073	0.007	0.006
Victivallales	0.017	0.021	0.000	0.000	0.000	0.000	0.010	0.017
YS2	0.010	0.017	0.017	0.021	0.000	0.000	0.003	0.006

	Time point							
Family	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Barnesiellaceae]	0.727	0.436	0.205	0.150	0.457	0.360	0.955	0.357
[Cerasicoccaceae]	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.006
[Mogibacteriaceae]	0.306	0.253	0.048	0.066	0.052	0.019	0.198	0.103
[Odoribacteraceae]	0.574	0.108	0.256	0.127	0.353	0.042	0.468	0.043
[Paraprevotellaceae]	0.247	0.329	0.846	1.341	0.000	0.000	0.007	0.006
[Tissierellaceae]	0.000	0.000	0.007	0.013	0.007	0.006	0.018	0.016
Actinomycetaceae	0.007	0.006	0.000	0.000	0.000	0.000	0.004	0.006
Alcaligenaceae	0.500	0.512	0.895	0.774	0.601	0.464	2.821	3.180
Bacteroidaceae	29.979	2.263	23.724	3.583	26.964	7.292	28.173	1.969
Bifidobacteriaceae	2.902	1.167	2.286	2.212	3.317	1.430	2.275	0.154
Carnobacteriaceae	0.011	0.019	0.000	0.000	0.000	0.000	0.011	0.010
Christensenellaceae	0.066	0.050	0.019	0.032	0.021	0.021	0.086	0.075
Clostridiaceae	1.252	0.190	0.841	0.620	0.396	0.408	0.644	0.470
Comamonadaceae	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.000
Coriobacteriaceae	0.914	0.331	0.422	0.344	0.927	0.350	0.944	0.562
Corynebacteriaceae	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000

Dehalobacteriaceae	0.000	0.000	0.000	0.000	0.004	0.006	0.003	0.006
Desulfovibrionaceae	0.910	0.830	1.613	1.892	2.704	2.494	2.582	1.754
Dethiosulfovibrionaceae	0.004	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Enterobacteriaceae	0.124	0.090	12.646	21.622	9.098	10.775	6.454	5.566
Enterococcaceae	0.011	0.019	0.035	0.060	1.095	1.870	0.769	1.303
Erysipelotrichaceae	2.990	2.332	12.701	16.916	2.999	3.923	2.253	3.472
Fusobacteriaceae	0.000	0.000	0.054	0.094	0.000	0.000	0.007	0.012
Helicobacteraceae	0.000	0.000	0.000	0.000	0.018	0.030	0.003	0.006
Lachnospiraceae	16.717	9.071	11.809	6.690	12.364	9.052	14.220	6.869
Lactobacillaceae	1.193	1.006	1.221	1.073	1.052	0.656	1.094	0.618
Leuconostocaceae	0.000	0.000	0.052	0.090	0.000	0.000	0.000	0.000
Methanobacteriaceae	0.053	0.093	0.000	0.000	0.004	0.006	0.000	0.000
Micrococcaceae	0.000	0.000	0.003	0.006	0.004	0.006	0.000	0.000
Oxalobacteraceae	0.004	0.006	0.000	0.000	0.003	0.006	0.003	0.006
Pasteurellaceae	0.004	0.006	0.000	0.000	0.007	0.012	0.014	0.006
Peptococcaceae	0.114	0.198	0.000	0.000	0.000	0.000	0.004	0.006
Peptostreptococcaceae	0.007	0.006	0.007	0.013	0.007	0.006	0.007	0.006
Phyllobacteriaceae	0.000	0.000	0.000	0.000	0.003	0.006	0.000	0.000
Porphyromonadaceae	2.140	0.686	1.200	1.266	1.753	0.850	1.998	0.314
Prevotellaceae	2.531	2.186	7.926	10.446	0.109	0.171	0.711	1.204
Pseudomonadaceae	0.000	0.000	0.000	0.000	0.007	0.012	0.000	0.000
Rikenellaceae	2.145	0.292	0.907	1.054	1.553	1.328	2.282	0.781
Ruminococcaceae	21.641	3.622	13.048	3.685	23.063	5.380	19.451	4.345
S24-7	0.476	0.824	0.526	0.688	2.068	3.075	0.379	0.656
Streptococcaceae	8.995	6.550	4.909	6.736	7.435	4.002	7.944	5.506
Turicibacteraceae	0.135	0.170	0.098	0.095	0.035	0.061	0.064	0.060
Veillonellaceae	2.302	0.540	1.619	0.475	1.434	1.020	3.132	0.810
Verrucomicrobiaceae	0.000	0.000	0.065	0.088	0.084	0.076	0.007	0.006
Victivallaceae	0.018	0.023	0.000	0.000	0.000	0.000	0.011	0.019

	Time point							
Genus	**T0**		**T5**		**T10**		**T24**	
	mean ± sd		**mean ± sd**		**mean ± sd**		**mean ± sd**	
[Eubacterium]	2.891	2.672	3.337	2.805	2.634	4.312	1.764	3.030
[Prevotella]	0.005	0.008	1.189	2.060	0.000	0.000	0.000	0.000
[Ruminococcus]	0.860	0.365	0.584	0.700	0.753	0.428	0.936	0.473
Acidaminococcus	0.069	0.120	0.092	0.159	0.216	0.373	1.694	2.308
Actinomyces	0.009	0.008	0.000	0.000	0.000	0.000	0.005	0.009

Adlercreutzia	0.034	0.048	0.000	0.000	0.107	0.130	0.175	0.177
Akkermansia	0.000	0.000	0.094	0.123	0.120	0.112	0.010	0.009
Anaerococcus	0.000	0.000	0.005	0.009	0.005	0.009	0.005	0.008
Anaerostipes	0.183	0.159	0.061	0.028	0.065	0.041	0.040	0.033
Anaerotruncus	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.017
Bacteroides	39.285	4.112	33.975	3.012	40.074	6.642	39.646	3.751
Bifidobacterium	3.759	1.424	3.170	3.090	4.867	1.710	3.196	0.179
Bilophila	0.761	1.168	2.248	2.519	4.190	4.342	3.279	2.793
Blautia	5.194	0.680	5.871	2.686	4.192	3.579	5.046	1.697
Bulleidia	0.000	0.000	0.000	0.000	0.437	0.757	0.000	0.000
Butyricimonas	0.321	0.208	0.098	0.072	0.092	0.091	0.207	0.123
Campylobacter	0.000	0.000	0.015	0.025	0.000	0.000	0.000	0.000
cc_115	0.023	0.022	0.169	0.294	0.032	0.055	0.010	0.017
Christensenella	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.009
Citrobacter	0.000	0.000	8.937	15.479	0.613	0.968	0.010	0.009
Clostridium	0.310	0.475	0.201	0.163	0.043	0.012	0.035	0.022
Collinsella	0.607	0.562	0.249	0.114	0.661	0.601	0.738	0.758
Coprobacillus	0.019	0.033	0.010	0.017	0.005	0.009	0.068	0.106
Coprococcus	5.349	5.488	2.541	2.363	4.164	4.637	3.322	2.540
Corynebacterium	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.000
Dehalobacterium	0.000	0.000	0.000	0.000	0.005	0.009	0.005	0.009
Delftia	0.000	0.000	0.005	0.009	0.000	0.000	0.000	0.000
Desulfovibrio	0.386	0.669	0.063	0.109	0.103	0.121	0.289	0.501
Dialister	1.940	1.719	0.707	1.137	1.586	2.199	1.353	1.577
Dorea	0.570	0.062	0.666	0.317	0.351	0.240	2.163	1.894
Eggerthella	0.005	0.009	0.019	0.034	0.000	0.000	0.005	0.009
Enterobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.009
Enterococcus	0.015	0.026	0.019	0.034	1.631	2.781	1.083	1.848
Epulopiscium	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.022
Erwinia	0.000	0.000	0.024	0.042	0.006	0.011	0.000	0.000
Escherichia	0.000	0.000	0.000	0.000	0.328	0.568	0.223	0.182
Faecalibacterium	4.294	1.990	3.150	0.792	3.810	1.869	3.206	0.336
Finegoldia	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.017
Flavobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fusobacterium	0.000	0.000	0.086	0.148	0.000	0.000	0.010	0.018
Granulicatella	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.009
Haemophilus	0.005	0.009	0.000	0.000	0.011	0.018	0.020	0.008
Helicobacter	0.000	0.000	0.000	0.000	0.016	0.027	0.005	0.009

Holdemania	0.019	0.022	0.065	0.051	0.062	0.075	0.024	0.042
Klebsiella	0.000	0.000	0.019	0.034	0.006	0.011	0.000	0.000
Lachnobacterium	0.014	0.025	0.045	0.078	0.310	0.537	0.015	0.026
Lachnospira	1.304	1.957	0.374	0.310	0.933	0.414	1.042	0.779
Lactobacillus	1.372	1.183	1.595	1.367	1.351	0.757	1.363	0.706
Lactococcus	0.000	0.000	0.020	0.023	0.011	0.010	0.000	0.000
Megasphaera	0.000	0.000	0.005	0.008	0.010	0.009	0.010	0.008
Mesorhizobium	0.000	0.000	0.000	0.000	0.005	0.008	0.000	0.000
Methanobrevibacter	0.068	0.118	0.000	0.000	0.006	0.011	0.000	0.000
Morganella	0.000	0.000	0.485	0.839	0.000	0.000	0.025	0.043
Odoribacter	0.432	0.345	0.269	0.107	0.443	0.061	0.452	0.194
Oscillospira	2.474	1.681	2.002	1.166	4.451	3.702	3.894	2.109
p-75-a5	0.000	0.000	0.005	0.009	0.006	0.011	0.015	0.025
Parabacteroides	2.781	0.813	1.672	1.660	2.738	1.628	2.795	0.352
Paraprevotella	0.259	0.448	0.075	0.130	0.000	0.000	0.005	0.009
Peptococcus	0.145	0.252	0.000	0.000	0.000	0.000	0.005	0.009
Peptoniphilus	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.009
Phascolarctobacterium	0.994	1.084	1.465	1.492	0.398	0.417	1.192	1.597
Plesiomonas	0.000	0.000	0.000	0.000	0.005	0.008	0.000	0.000
Porphyromonas	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.008
Prevotella	3.251	2.805	12.241	16.758	0.186	0.296	0.971	1.641
PSB-M-3	0.000	0.000	0.005	0.008	0.000	0.000	0.000	0.000
Pseudomonas	0.000	0.000	0.000	0.000	0.009	0.016	0.000	0.000
Roseburia	0.847	1.298	0.686	0.974	0.317	0.282	0.215	0.300
Rothia	0.000	0.000	0.005	0.008	0.005	0.009	0.000	0.000
Ruminococcus	6.533	3.877	2.474	1.601	3.314	2.790	3.907	0.555
Serratia	0.005	0.009	0.635	1.087	1.925	3.335	0.000	0.000
Slackia	0.018	0.031	0.005	0.009	0.057	0.075	0.055	0.052
SMB53	0.186	0.211	0.059	0.051	0.042	0.074	0.127	0.151
Streptococcus	11.595	8.374	6.646	8.938	11.282	6.145	11.118	7.632
Sutterella	0.616	0.714	1.343	1.186	0.839	0.615	3.893	4.309
Trabulsiella	0.000	0.000	0.005	0.008	0.000	0.000	0.005	0.009
Turicibacter	0.177	0.220	0.133	0.128	0.061	0.105	0.090	0.082
Veillonella	0.000	0.000	0.078	0.110	0.095	0.164	0.153	0.239

Appendix B

Supplementary Tables Chapter 3

Table A1. Wilcoxon rank-sum test results for each alpha-diversity calculated on the number of observed OTUs, the Chao1 index and the Shannon entropy index

	Metric	***p* value***
Placebo A vs Placebo B	Observed	0.5167
	Chao1	0.7209
	Shannon	0.7065
Placebo A vs VSL#3 A	Observed	0.5167
	Chao1	0.7209
	Shannon	0.3427
Placebo A vs VSL#3 B	Observed	0.7546
	Chao1	0.7209
	Shannon	0.3427
Placebo B vs VSL#3 A	Observed	0.5167
	Chao1	0.7209
	Shannon	0.3427
Placebo B vs VSL#3 B	Observed	0.5167
	Chao1	0.7209
	Shannon	0.3427
VSL#3 A vs VSL#3 B	Observed	0.5167
	Chao1	0.7209
	Shannon	1.000

*Bonferroni corrected p-values

Table A2. Mean relative abundance (%) ± standard deviation (SD) of bacterial OTUs in placebo subjects at time zero (Placebo A) and after treatment (Placebo B).

Species	**Placebo A mean ± sd**		**Placebo B mean ± sd**	
Ruminococcu bromii	25.3692	15.8105	18.8758	12.2916
Bifidobacterium bifidum	19.0553	26.7762	11.0522	9.1910
Bacteroides gadei	10.3927	10.0760	9.7545	10.1620
Bifidobacterium pseudolongum	10.3860	7.3847	9.7613	6.3789
Akkermansia	9.5480	10.5999	6.8879	11.5825
Collinsella stercoris	6.4283	6.6761	5.6622	2.7985
Bacteroides plebeius	4.5248	5.3153	2.9789	3.7003
Bacteroides eggerthii	1.8163	0.9764	1.5760	0.8545
Veillonella parvula	1.5848	2.1582	1.7724	1.7600
Prevotella aureus	1.5302	2.6181	1.7761	3.7807

Blautia producta	1.3357	0.7530	1.2331	0.7553
Faecalibacterium prausnitzii	1.2856	1.6195	0.1996	0.2595
[Eubacterium] adhaesivum	1.2142	1.5265	0.5007	0.9200
Eggerthella caccae	1.0751	1.5655	0.3553	0.3230
Haemophilus johnsonii	0.9612	1.9224	0.0716	0.1317
Coprococcus formicigenerans	0.9335	1.0997	6.7445	11.3838
Parabacteroides copri	0.6916	0.4843	0.5158	0.1747
Blautia eutactus	0.6084	0.6283	0.2746	0.2125
Bacteroides fragilis	0.3062	0.6303	2.4452	5.6226
Bacteroides ovatus	0.2601	0.1971	0.4260	0.5797
[Eubacterium] dolichum	0.1795	0.3714	0.0127	0.0293
Ruminococcus noxia	0.1389	0.1176	0.5152	0.6238
[Eubacterium] cylindroides	0.1313	0.2717	0.4121	0.9487
Streptococcus sobrinus	0.1183	0.2411	0.0319	0.0576
Roseburia gnavus	0.0255	0.0203	0.0290	0.0219
Aggregatibacter parainfluenzae	0.0211	0.0437	0.0000	0.0000
Staphylococcus equorum	0.0170	0.0214	0.0360	0.0302
Clostridium obeum	0.0142	0.0181	0.0051	0.0087
Rothia adolescentis	0.0106	0.0097	0.0156	0.0223
Oxalobacter coli	0.0080	0.0165	0.0125	0.0288
Lactobacillus acidilactici	0.0057	0.0092	1.3403	2.6811
Pyramidobacter muciniphila	0.0045	0.0094	0.0322	0.0675
Pediococcus garvieae	0.0039	0.0054	0.0850	0.1909
Staphylococcus mucosae	0.0036	0.0048	0.0161	0.0372
Pseudomonas anthropi	0.0015	0.0032	0.0017	0.0039
Selenomonas dispar	0.0015	0.0031	0.0000	0.0000
Veillonella biforme	0.0015	0.0031	0.0021	0.0031
Rothia mucilaginosa	0.0012	0.0025	0.0096	0.0196
Lactococcus anginosus	0.0012	0.0025	0.0057	0.0089
Methylobacterium formigenes	0.0012	0.0025	0.0011	0.0026
Escherichia morganii	0.0012	0.0025	0.0073	0.0114
Dorea faecis	0.0012	0.0024	0.0000	0.0000

Table A3. Mean relative abundance (%) ± standard deviation (SD) of bacterial OTUs in VSL#3 treated subjects at time zero (VSL#3 A) and after treatment (VSL#3 B).

Species	**VSL#3 A mean ± sd**		**VSL#3 B mean ± sd**	
Ruminococcus bromii	24.9425	18.3981	10.5788	9.6048
Bifidobacterium bifidum	11.6391	9.0232	16.1874	13.9684

Bifidobacterium pseudolongum	11.5582	6.6043	34.5783	31.3134
Akkermansia	9.3796	16.1058	0.0057	0.0033
Veillonella parvula	8.1652	8.5054	2.2412	2.2050
Collinsella stercoris	7.7141	6.5915	8.3773	7.1389
Bacteroides gadei	7.5445	8.9452	3.1893	4.7841
[Eubacterium] cylindroides	3.9125	6.7265	0.0000	0.0000
Bacteroides plebeius	2.7361	3.2363	0.8699	0.7481
Bacteroides fragilis	2.1284	3.6592	2.5390	4.0624
Bacteroides eggerthii	1.6310	2.1336	1.0557	1.0652
Lactobacillus acidilactici	1.4687	1.6012	0.7183	0.9687
Parabacteroides copri	1.4445	1.2669	0.4160	0.4234
Blautia eutactus	0.9905	1.1008	2.4534	3.4143
Ruminococcus noxia	0.9781	1.1510	2.1578	2.3750
[Eubacterium] adhaesivum	0.8083	1.1220	4.0055	2.9539
Eggerthella caccae	0.6680	0.7203	0.6634	0.6062
Bacteroides ovatus	0.5681	0.8540	2.2723	2.9588
Blautia producta	0.5325	0.5243	0.3440	0.2657
Pediococcus garvieae	0.4457	0.7610	3.3620	5.0775
Faecalibacterium prausnitzii	0.2422	0.2082	0.4714	0.4936
Clostridium obeum	0.1735	0.2853	0.0012	0.0019
Coprococcus formicigenerans	0.0499	0.0798	0.0000	0.0000
Lactobacillus ruminis	0.0466	0.0801	0.0070	0.0094
Roseburia gnavus	0.0359	0.0235	0.0114	0.0092
Morganella segnis	0.0331	0.0569	0.0020	0.0032
Haemophilus johnsonii	0.0310	0.0441	0.0317	0.0508
Bifidobacterium aerofaciens	0.0268	0.0405	0.9886	1.5825
Streptococcus sobrinus	0.0226	0.0280	0.0250	0.0251
Rothia adolescentis	0.0215	0.0232	0.0126	0.0092
Dysgonomonas distasonis	0.0101	0.0173	0.0000	0.0000
Escherichia morganii	0.0086	0.0149	0.0217	0.0214
Dorea faecis	0.0075	0.0108	0.0000	0.0000
Veillonella biforme	0.0053	0.0092	0.0000	0.0000
Aggregatibacter parainfluenzae	0.0053	0.0092	0.0000	0.0000
Staphylococcus equorum	0.0043	0.0051	0.0165	0.0248
[Eubacterium] dolichum	0.0033	0.0057	0.0137	0.0220
Lactobacillus reuteri	0.0029	0.0050	0.0000	0.0000
Jonquetella piscolens	0.0028	0.0049	0.0000	0.0000
Clostridium perfringens	0.0027	0.0046	0.0018	0.0019
Rothia mucilaginosa	0.0025	0.0028	0.0062	0.0071
Streptococcus hiranonis	0.0022	0.0038	0.0120	0.0192

Acinetobacter veronii	0.0015	0.0025	0.0009	0.0014
Selenomonas dispar	0.0013	0.0023	0.0000	0.0000
Prevotella aureus	0.0011	0.0019	0.0011	0.0018

Table A4. Mean relative abundance (%) ± standard deviation (SD) of bacterial OTUs at phylum and genus levels in placebo subjects at time zero (Placebo A) and after treatment (Placebo B).

Phylum	**Placebo A mean ± sd**		**Placebo B mean ± sd**	
Firmicutes	62.8911	31.3035	66.3142	28.2484
Bacteroidetes	18.5002	27.3994	15.6216	32.4831
Actinobacteria	13.7728	26.2122	14.9279	21.7833
Verrucomicrobia	2.7280	7.3946	1.6947	10.7678
Proteobacteria	2.0865	7.6064	1.4003	6.4096
Euryarchaeota	0.0091	0.0457	0.0179	0.1559
TM7	0.0044	0.0122	0.0021	0.0128
Cyanobacteria	0.0031	0.0048	0.0013	0.0038
[Thermi]	0.0014	0.0036	0.0029	0.0142
Synergistetes	0.0013	0.0065	0.0079	0.0627
Lentisphaerae	0.0010	0.0051	0.0000	0.0000
Fusobacteria	0.0009	0.0046	0.0045	0.0390
FBP	0.0003	0.0015	0.0000	0.0000
Tenericutes	0.0000	0.0000	0.0047	0.0195

Genus	**Placebo A mean ± sd**		**Placebo B mean ± sd**	
Bacteroides	18.8303	18.0160	16.2303	15.2853
Bifidobacterium	15.5980	16.9148	17.8382	12.6348
Coprococcus	12.2004	7.6454	10.7919	9.4874
Blautia	11.3537	6.3178	12.1481	6.4445
Faecalibacterium	10.2037	8.6650	6.5619	6.4149
Streptococcus	6.1190	12.5240	9.9395	12.5188
Ruminococcus	6.0894	2.2079	6.1898	2.9898
Akkermansia	3.8415	5.8101	2.3945	6.0448
Collinsella	2.5879	3.6585	1.9904	1.4659
Dialister	2.2178	3.2700	2.8713	4.1196
[Ruminococcus]	2.1559	1.9275	1.3356	1.5271
Dorea	1.3217	1.0927	2.0435	1.0270
Oscillospira	1.0387	0.9176	1.0612	0.4872
Parabacteroides	0.9147	0.8331	0.6094	0.6688
[Eubacterium]	0.7956	1.3935	0.6138	1.0550
Veillonella	0.6385	1.1843	0.6169	0.9180

Prevotella	0.6171	1.4337	0.6202	1.9786
Eggerthella	0.4324	0.8580	0.1235	0.1686
Haemophilus	0.4090	1.1169	0.0252	0.0699
Clostridium	0.3792	0.3548	0.4747	0.5994
Odoribacter	0.2923	0.4962	0.1514	0.1573
Sutterella	0.2560	0.4379	0.0472	0.0852
Turicibacter	0.2248	0.3364	0.1508	0.2677
Adlercreutzia	0.1857	0.3732	0.1212	0.2495
Lachnospira	0.1625	0.1622	0.1868	0.2939
Anaerostipes	0.1332	0.1416	0.2979	0.2259
Megamonas	0.1044	0.2944	0.1193	0.4122
Paraprevotella	0.0941	0.1700	0.2078	0.5878
Bilophila	0.0929	0.1802	0.0328	0.0430
Desulfovibrio	0.0891	0.1829	0.0941	0.3177
Roseburia	0.0798	0.0828	0.0772	0.1068
Phascolarctobacterium	0.0695	0.0999	0.0757	0.1967
Coprobacillus	0.0501	0.0647	0.0330	0.0467
Granulicatella	0.0369	0.0746	0.0198	0.0155
Actinomyces	0.0347	0.0398	0.0506	0.0766
Enterococcus	0.0314	0.0475	0.5229	1.6517
Lactobacillus	0.0275	0.0552	1.9360	4.9056
Megasphaera	0.0275	0.0761	0.0007	0.0023
Lachnobacterium	0.0225	0.0506	1.0043	3.4488
Anaerotruncus	0.0223	0.0385	0.0048	0.0074
Aggregatibacter	0.0200	0.0565	0.0000	0.0000
Anaerofustis	0.0193	0.0286	0.0664	0.1793
Holdemania	0.0160	0.0109	0.0084	0.0068
Citrobacter	0.0152	0.0428	0.0000	0.0000
Lactococcus	0.0135	0.0340	0.0040	0.0073
Corynebacterium	0.0131	0.0292	0.0079	0.0106
Methanobrevibacter	0.0128	0.0359	0.0253	0.0875
SMB53	0.0123	0.0189	0.0091	0.0130
Hymenobacter	0.0114	0.0116	0.0127	0.0217
Slackia	0.0109	0.0308	0.0087	0.0299
Staphylococcus	0.0083	0.0128	0.0193	0.0236
cc_115	0.0071	0.0144	0.0013	0.0045
Leuconostoc	0.0059	0.0166	0.0071	0.0112
Acidaminococcus	0.0050	0.0140	0.0564	0.1324
Rothia	0.0048	0.0065	0.0088	0.0217
Sphingomonas	0.0035	0.0038	0.0008	0.0027

Oxalobacter	0.0032	0.0090	0.0043	0.0150
Butyricimonas	0.0031	0.0056	0.0203	0.0599
Bulleidia	0.0029	0.0039	0.0003	0.0010
Dehalobacterium	0.0026	0.0040	0.0062	0.0134
Deinococcus	0.0020	0.0028	0.0041	0.0080
Pyramidobacter	0.0018	0.0051	0.0112	0.0352
Pediococcus	0.0016	0.0029	0.0301	0.0993
Serratia	0.0015	0.0041	0.0000	0.0000
Peptostreptococcus	0.0014	0.0028	0.0023	0.0024
Mesorhizobium	0.0014	0.0026	0.0013	0.0022
Peptoniphilus	0.0014	0.0027	0.0057	0.0196
Atopobium	0.0013	0.0025	0.0048	0.0081
Fusobacterium	0.0013	0.0036	0.0063	0.0219
Anaerococcus	0.0012	0.0035	0.0032	0.0089
Acinetobacter	0.0011	0.0020	0.0009	0.0030
Mogibacterium	0.0011	0.0020	0.0000	0.0000
Kineococcus	0.0011	0.0020	0.0026	0.0063

Table A5. Mean relative abundance (%) ± standard deviation (SD) of bacterial OTUs at phylum and genus levels in VSL#3 treated subjects at time zero (VSL#3 A) and after treatment (VSL#3 B).

Phylum	**VSL#3 A mean ± sd**		**VSL#3 B mean ± sd**	
Firmicutes	61.4592	32.2273	59.9439	38.9242
Bacteroidetes	17.8785	29.7245	6.0176	12.9508
Actinobacteria	16.2091	19.8766	29.4074	29.1935
Verrucomicrobia	2.3289	12.4278	0.0020	0.0045
Proteobacteria	2.0811	5.5745	4.5922	18.7261
Fusobacteria	0.0302	0.1365	0.0240	0.1431
TM7	0.0068	0.0134	0.0020	0.0073
Cyanobacteria	0.0037	0.0083	0.0009	0.0025
[Thermi]	0.0017	0.0073	0.0038	0.0125
Synergistetes	0.0007	0.0037	0.0000	0.0000
Euryarchaeota	0.0000	0.0000	0.0054	0.0323
FBP	0.0000	0.0000	0.0008	0.0030

Genus	**VSL#3 A mean ± sd**		**VSL#3 B mean ± sd**	
Bifidobacterium	21.9571	13.0528	34.8223	17.1220
Bacteroides	15.8570	11.4011	6.7008	5.5927
Streptococcus	9.4927	11.6893	10.2088	17.8602
Lactobacillus	8.8494	9.4110	2.0448	1.6432

Blautia	7.4817	7.7956	6.0312	5.5939
Coprococcus	5.4913	4.4630	5.3584	4.9365
Veillonella	4.7068	4.2111	1.9613	2.3610
Faecalibacterium	4.5059	5.2907	8.8974	12.4892
Ruminococcus	3.2100	3.9937	8.0558	8.7221
Akkermansia	2.8756	5.9429	0.0026	0.0025
Pediococcus	2.6784	5.2091	1.6204	3.7394
Collinsella	2.2235	2.4764	3.5048	5.3968
[Eubacterium]	1.6743	2.3412	2.6431	2.1426
Dorea	1.3677	1.1853	0.8406	0.7507
Peptostreptococcus	1.0984	2.2216	0.0014	0.0035
Sutterella	0.9078	1.4260	0.1860	0.1751
Acidaminococcus	0.8273	1.4828	0.2388	0.3900
[Ruminococcus]	0.7645	0.6234	1.1600	1.8281
Parabacteroides	0.6570	0.7987	0.6016	0.7170
Coprobacillus	0.4512	0.9252	0.5170	0.8028
Dialister	0.3793	0.3824	0.2858	0.6954
Phascolarctobacterium	0.3259	0.4857	0.3534	0.5515
Lactococcus	0.3205	0.6327	0.6061	1.4816
Oscillospira	0.3186	0.2884	0.4435	0.5043
Enterococcus	0.2912	0.4141	1.0913	1.5490
Clostridium	0.2337	0.3742	0.2889	0.4247
Eggerthella	0.2133	0.2649	0.3241	0.4444
Anaerostipes	0.1709	0.2383	0.2586	0.3751
Roseburia	0.0951	0.0859	0.0832	0.0678
Adlercreutzia	0.0703	0.1313	0.0928	0.1577
Actinomyces	0.0633	0.0604	0.0373	0.0242
Fusobacterium	0.0383	0.0648	0.0318	0.0778
Bilophila	0.0355	0.0702	0.0523	0.0748
Megasphaera	0.0352	0.0637	0.0037	0.0091
Granulicatella	0.0334	0.0231	0.0524	0.0628
Anaerotruncus	0.0303	0.0627	0.0000	0.0000
Leuconostoc	0.0291	0.0404	0.0015	0.0026
Turicibacter	0.0262	0.0541	0.0395	0.0539
Atopobium	0.0218	0.0407	0.0527	0.1151
Lachnospira	0.0186	0.0236	0.0961	0.2006
Odoribacter	0.0142	0.0293	0.0064	0.0109
Anaerofustis	0.0134	0.0136	0.0197	0.0298
Finegoldia	0.0127	0.0139	0.0069	0.0107
Rothia	0.0110	0.0094	0.0091	0.0108

Morganella	0.0102	0.0210	0.0010	0.0024
Prevotella	0.0092	0.0092	0.0038	0.0092
Peptoniphilus	0.0077	0.0097	0.0048	0.0080
Citrobacter	0.0076	0.0100	0.0112	0.0127
SMB53	0.0075	0.0116	0.0059	0.0070
Anaerococcus	0.0066	0.0080	0.0038	0.0052
Corynebacterium	0.0065	0.0064	0.0045	0.0075
Lachnobacterium	0.0061	0.0101	0.0004	0.0010
Selenomonas	0.0057	0.0108	0.0000	0.0000
Mesorhizobium	0.0049	0.0080	0.0008	0.0013
Staphylococcus	0.0045	0.0064	0.0083	0.0181
Porphyromonas	0.0039	0.0070	0.0000	0.0000
Dysgonomonas	0.0031	0.0064	0.0000	0.0000
Butyricimonas	0.0030	0.0063	0.0484	0.1062
Escherichia	0.0026	0.0055	0.0104	0.0158
Mogibacterium	0.0025	0.0035	0.0000	0.0000
Pseudoramibacter_Eubacterium	0.0022	0.0030	0.1648	0.4019
Hymenobacter	0.0022	0.0030	0.0083	0.0067
Comamonas	0.0022	0.0045	0.0000	0.0000
Deinococcus	0.0021	0.0035	0.0050	0.0068
Peptococcus	0.0020	0.0041	0.0013	0.0031
Slackia	0.0016	0.0034	0.0009	0.0022
Aggregatibacter	0.0016	0.0034	0.0000	0.0000
Campylobacter	0.0016	0.0017	0.0005	0.0013
Haemophilus	0.0014	0.0021	0.0156	0.0373
Kineococcus	0.0011	0.0015	0.0009	0.0014
Parvimonas	0.0011	0.0015	0.0011	0.0016
Christensenella	0.0010	0.0021	0.0003	0.0008
Holdemania	0.0010	0.0021	0.0147	0.0282

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